THE RESPONSE OF TISSUE TO TOTAL BODY IRRADIATION*

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It has been fairly well established that, provided ionization within tissue is achieved, the biologic effects brought about by any of the several types of ionizing radiations are essentially similar.[1-4] Thus the effect produced by penetrating external radiations is similar to the effect of internally deposited materials, provided equal amounts of roentgen units or roentgen equivalents are delivered to the tissue.

Any cell will be destroyed if it receives a sufficiently large dose of ionizing radiations. However, for reasons unknown, there are marked differences in the sensitivity of the various cells of a given species to irradiation; and, even among cells of a single type in different stages of development there are marked differences in radiosensitivity. In general in a given cell series, the cells in the early stages of mitotic division and blast cells are radiosensitive while mature cells are more radioresistant.

It is of special interest that reticular cells of lymph nodes and bone marrow are relatively radioresistant. This fact has not been emphasized in the literature, but seems to be quite clear from histopathologic observations. For instance, in swine that have received heavy doses of total body irradiation, reticular cells in the lymph nodules are prominent among the surviving cells. The radioresistance of reticular cells plays an important part in the recovery of borderline cases that receive heavy doses.

It has been reported that the radioresistance of various mature cells increases in approximately the following order: lymphocytes, germ cells, granulocytes, epithelium, endothelium, muscle, connective tissue,

bone, and nerve cells.[5,6] In these cells all degrees of injury may be observed, depending on the stage of development of the cells at the time of injury, the degree of ionization accomplished, and probably other factors.

The problem of interpretation of biologic response to radiation is further complicated by differences in species sensitivity. Guinea-pigs, for instance, are radiosensitive while rats are relatively radioresistant. Goats and swine fall somewhere between the two, within what is estimated as the sensitivity range of man.

Finally, it is important to grasp the concept of total body irradiation in contrast to localized irradiation. An amount of radiation that is well tolerated when given locally might be sufficient to kill if administered simultaneously to the total body. For instance, it is estimated that 300 to 600 r. of total body irradiation is the range of lethal dosage for man, yet doses up to 10,000 r. may be given to a small tumor with no general reaction whatsoever; and doses up to 400 r. may be given to an area up to 20 by 20 cm. with no more than transient radiation sickness characterized by nausea and perhaps vomiting.

MATERIALS AND METHODS

One group of swine discussed in this paper was exposed to total body irradiation from atomic bomb explosions at Bikini in the summer of 1946. The Naval Medical Research Section of Joint Task Force One placed the animals on target ships at various distances from the bomb explosion. The test positions afforded degrees of shielding from ionizing radiations varying from very little to the amount of protection supplied by 6 or 8 inches of steel. The animals were recovered as soon after the tests as practicable and a few survivors are still under observation. The biologic experimental design is more fully described in other reports.[7,8]

The second group of swine discussed in the present report was studied separately at the Naval Medical Research Institute.[9,10] These swine were subjected to radiations from a 1000 kv., 3 ma. x-ray source with no added filter at 1 m. distance. The experimental design permitted the delivery of measurable amounts of penetrating x-rays to the total body of 4 swine at a time at the rate of 30 r. per minute. The 50 per cent depth dose, measured in a medium of rice and sodium bicarbonate, was delivered to the approximate mid-vertical plane of the swine. In an effort to achieve uniform dosage throughout the animals, they were irradiated with the same dose on each lateral aspect in succession. The $LD_{50}/30$ days was established as 275 r. measured in air, administered bilaterally[9] and the tissue responses to the different doses were studied.[10]

RESULTS

Mortality

The period between exposure to ionizing irradiation and death was found to be roughly proportional to the size of the dose. Some of the animals exposed at Bikini died soon after the explosion, but owing to the experimental conditions most of them were not recovered for 1 to 5 days after the test. The animals which were dead on the target ships at the time of recovery generally had undergone too extensive autolysis to be studied profitably. However, the animals which were recovered alive could be studied carefully. Deaths occurred in these animals after a latent period of 1 to 29 days. The single death that occurred after the 30th day was that of an animal in which leukopenia had persisted from the time of irradiation and was the result of pneumonic involvement. The other late deaths probably were not caused by irradiation damage.

All but one of the swine irradiated with million volt x-rays died within 30 days or recovered.[9] The one survivor, which subsequently died on the 91st day after irradiation, had a consistently low white blood cell count and developed pneumonia. This swine and the Bikini swine cited are the only deaths in the two experiments so far which might be classed as delayed radiation deaths.

Gross Pathologic Findings

The lesions in swine exposed to lethal amounts of ionizing radiations were of three main types: hemorrhage, necrosis, and secondary infections. The similarity between the gross lesions produced by ionizing radiation from the release of atomic energy (Figs. 1, 3, 5, 7, 9, and 11) and those produced by exposure to million volt x-rays (Figs. 2, 4, 6, 8, 10, and 12) was manifest. Hemorrhages occurred as petechiae, ecchymoses, and sometimes as small hematomas. All parts of the body and all types of tissues were involved. Extravasation of blood into tissue spaces was widespread throughout the body and accounted for considerable hematic loss, but a much more voluminous loss occurred via the gastro-intestinal and urinary tracts. Surface erosions and ulcerations in the gastro-intestinal tract (Figs. 7 to 10) were gross bleeding areas which fostered external blood loss. The source of the considerable bleeding into the kidney pelves (Figs. 11 and 12) was not obvious in the sense that no ulcerations or bleeding points could be seen grossly. This hemorrhage appeared clinically as hematuria and was a sign of severe radiation illness.

The macroscopic manifestations of necrosis appeared as ulceration in

mouth, tonsils (Figs. 3 and 4), stomach (Figs. 7 and 8), and the small and large intestines. In swine, ulcers occurred more frequently in the large than in the small intestine. In all parts of the gastro-intestinal tract ulceration occurred more frequently in the Bikini animals than in those that died from million volt x-radiation, but in the mouth and tonsils, ulcers were equally prevalent in the two groups. A striking feature of the ulceration in both groups was the absence of purulent reaction. The ulcers were shallow, clearly demarcated, hemorrhagic, and free of pus.

Swine that survived the hemorrhagic and ulcerative lesions produced by irradiation frequently succumbed to secondary infections to which they were more than normally susceptible because of leukopenia, lowered resistance, anemia, and bone marrow damage. Pneumonia, usually of bilateral lobular distribution in hemorrhagic edematous lungs, was often a contributory cause of death in irradiated animals. In borderline cases which might otherwise have survived, the development of pneumonia was fatal. Other infections were uncommon. One animal had pericarditis with pneumonia and one died from a generalized gas bacillus infection. There were no generalized skin abscesses.

Histopathologic Findings

The histopathologic features were similar in the two experimental groups. Those of the x-irradiated animals will be published elsewhere.[10] The present report deals solely with the histopathologic findings in the Bikini swine. The microscopic tissue changes which will be discussed were observed in swine which died within 14 days after exposure to total body irradiation well over 1000 r. This dose is well in excess of the absolute lethal dose.

It is assumed that the mechanism of action of ionizing radiation is to produce ionization within the cytoplasm and/or nucleus of the cell, thus disturbing its physiologic functions or chemical balance. The injury may extend to irreparable damage or only to partial damage from which total or partial recovery is possible. The greatest morphologic changes are found in the more radiosensitive cells while the radioresistant cells show little, if any, alteration following exposure to ionizing radiation.

The bone marrow and lymphoid elements underwent changes with small doses of total body radiation. When, as reported here, lethal amounts of radiation were used and the terminal picture was described, very extensive damage to the tissue was seen (Figs. 13 and 14). In addition to extravasation of blood, which occurred at random throughout the body, the outstanding feature of irradiated marrow and lymph nodes was the reduced cellularity. In bone marrow, only scattered islands of

hematopoietic tissue remained and cell reproduction in these islands often appeared stagnant. It was not uncommon to find intact only fat cells, a few clumps of erythrocytes, lining cells of blood vessels, and dispersed individual reticular cells and myelocytes (Fig. 15). Blast cells were very difficult to find in the bone marrow of animals that died of ionizing radiation injuries. The stroma assumed an opaque ground-glass appearance.

In lymph nodes, the lymphoid cells, particularly the small lympho-cytes, were depleted. This was particularly noticeable in the lymph nodules in which the reticular cells assumed relative prominence in the absence of the normal lymphocyte population (Fig. 16). The large lymphocytes did not appear to be as markedly depleted as the small lymphocytes. It is interesting that vacuolation of cytoplasm or nucleus in recognizable lymphocytes or monocytes was not found. Evidently, the irradiation effect on these cells, when great enough to cause any morphologic alteration, caused pyknosis, karyorrhexis, and rapid de-struction. In some of the nodes free (circulating) macrophages were increased, probably through the process of mobilization of existing fixed (tissue) macrophages. Erythrophagocytosis (Fig. 17) and an abundance of blood pigment granules, both phagocytized and not phagocytized (Fig. 18), were commonly observed. Dilatation of the lymph sinuses and edema of the pulp usually were present. The margins of the dilated sinuses tended to undergo coagulation necrosis (Figs. 19 and 20). There was no cellular response, either granulocytic or lymphoid, to the necrosis, but the lymph tended to coagulate on the necrotic lining cells of the sinus. The lacy beaded networks of fibrin, which were formed, partially occluded the sinus. The collagen in the fibers of the stromal network underwent a hyaline change and became thickened and prominent.

Lymphoid tissue in other parts of the body, particularly in the spleen (Fig. 21), the tonsil (Fig. 22), the thymus, and the intestine, showed changes similar to those described in the lymph nodes. When the dose of ionizing radiations was great enough to cause death within a few days, necrosis and reduction in numbers of the lymphocytes were seen. On the other hand, when the animal survived 10 days or longer, the frag-mented and pyknotic lymphocytes had largely disappeared leaving fairly normal-appearing large and small lymphocytes in reduced numbers.

Marked congestion of the dilated blood sinuses in the spleen was an almost constant feature. This was associated after a few days with increased deposits of blood pigment and erythrophagocytosis. Lympho-poiesis was never increased and usually appeared depressed. There was no evidence of hematopoiesis in other cell lines.

In addition to the reduction in the diffuse and aggregated lymphoid elements in the tonsils, epithelial ulceration and follicular necrosis were common (Fig. 23). The necrosis did not elicit a granulocytic or lymphocytic response and seemed to occur in random follicles with no apparent reason for localization in the affected structures.

The same random location of lesions was found for mucosal ulceration in the stomach and intestine. Mucosal ulcers were more common in the large intestine than in the small intestine, and were more common in either of these locations than in the stomach or esophagus. The intestinal ulcers often were covered with a necrotic membrane (Fig. 24) and were sharply demarcated from the adjacent non-ulcerated mucosa (Fig. 25). Some of the vessels beneath the ulcers were thrombosed (Fig. 26), but this was not a constant finding. What part, if any, thrombosis played in producing ulceration can only be surmised. Vessels that did not contain thrombi were dilated and congested. Thrombosis was fairly constant and the resulting anoxia in the tissue supplied by these vessels probably materially influenced the extent of the necrosis. The submucosa usually was edematous and the cellular components were reduced in number. Large and small lymphocytes appeared to be slightly reduced in numbers in the edematous lamina propria and submucosa, but the lymphoid aggregations corresponding to Peyer's patches were markedly depleted of cells. Plasma cells were numerous and of normal appearance. The connective tissue cells of the submucosa sometimes had degenerated in a bizarre manner (Fig. 27). The affected cells became swollen and vacuolated. The collagen became an edematous, fibrillar, lacy network which spread aimlessly in the immediate vicinity.

Occasional hemorrhages occurred in the muscle and subserosa of the stomach and intestine, but otherwise these layers appeared to be uninvolved. Ulcers in the stomach were fewer, but larger and more hemorrhagic, than those in the intestine.

Evidence of damage less severe than ulceration was found in the glandular epithelium of both stomach and intestines. It took the form of distortion, variation in size, and vacuolization of the cells (Fig. 28). The differences in cell structure were apparent within the same gland as well as from gland to gland.

In the lungs, congestion of the vessels in the alveolar septa and extravasation of edema fluid into the alveoli were observed in most of the animals. The edema fluid sometimes was clear and homogeneous, but more often it contained fibrin, macrophages, and/or erythrocytes (Figs. 29 and 30). In some swine the lesions were more advanced and consisted of focal areas of necrosis and hemorrhage. A few macrophages

sometimes were seen in these areas but otherwise there was no white blood cell response to the lesions. The two features which characterized ionizing radiation injury of the lungs were (a) the great variability of the lesions in the same lung, ranging from congestion and edema to necrosis, and (b) the absence of cellular infiltration.

Some of the bronchioles contained a few desquamated epithelial cells and edema fluid, but others in the same lung appeared normal.

Focal areas of hemorrhage and necrosis were seen in the kidneys but with less than expected frequency (Fig. 31). The glomeruli appeared to be unharmed. The loops of Henle and collecting tubules were rarely involved, but the proximal and distal convoluted tubules seemed to be, to an equal degree, slightly more vulnerable to radiation injury. However, both the glomerular and tubular epithelia were highly radioresistant. Hemorrhages occurred along the course of the interlobular vessels but tended to remain localized.

The epithelium of the calyces, the pelvis, the ureters, and the bladder was more radiosensitive (Fig. 32) than that of the tubules. Congestion and hemorrhage in the epithelial and subepithelial layers were frequently observed and often were associated with vacuolization, desquamation, and necrosis of the epithelial cells. Hematuria, a common clinical observation in the heavily irradiated swine, probably was the result of lesions in the epithelium and lamina propria of the renal pelvis, ureter, and bladder. Lesions in the kidney parenchyma did not appear to contribute to the hematuria in any of the swine studied.

The male swine irradiated at Bikini were sexually immature. The normal seminiferous tubule in swine 3 to 4 months old contains only one or two rows of epithelium which consists of numerous primitive stem cells, the so-called indifferent cells, and a few spermatogonia and rare Sertoli cells. The epithelium is in the resting stage and spermatocytogenesis is not taking place. The testes of the Bikini swine that received a lethal dose of total body irradiation lost most of their spermatogonia; in fact, it was difficult to find these cells at all in many cases (Fig. 33). The indifferent cells of the seminiferous tubules exhibited no structural alterations after irradiation. Amorphous cellular débris filled the lumina of the tubules. The Sertoli cells and the interstitial tissue, except for scattered small hemorrhages, appeared unaltered by irradiation.

The sows at 3 to 4 months of age were more mature than the boars of the same age. This comment is based on the fact that an occasional corpus luteum was seen in the ovaries of 4-months-old sows while in the boars of comparable age there was no evidence of spermatogenesis. After lethal doses of total body irradiation the number of atretic follicles

was increased (Fig. 34). The chromatin in the nuclei of some of the
ova was clumped. Other ova were undergoing karyolysis or had dis-
appeared entirely. However, many ova showed no morphologic alter-
ation and presumably remained fertile. The stroma of the ovary became
edematous but the cells were unaltered. Hemorrhages in the ovary were
rare.

Vacuolation of nuclei and cytoplasm occurred in the epidermis, par-
ticularly in the basal layers (Fig. 35). In some nuclei the chromatin
became clumped and assumed bizarre configurations. The dermis became
edematous and the collagen underwent hyaline changes. The capillaries
of the dermis often were congested or plugged with agglutinative thrombi.
In the hair follicles the normally clearly defined layers of epithelium lost
their definition and tended to merge with one another (Fig. 36). The
sebaceous and sweat glands of the skin usually appeared unchanged.

The liver, pancreas, thyroid gland, adrenals, salivary glands, epi-
didymis, fallopian tubes, uterus, smooth muscle, striated muscle, cardiac
muscle, bone, cartilage, and nerve fibers rarely showed any evidence of
injury. Occasionally, hemorrhages occurred in the liver, adrenal gland,
and muscle, but in the other radioresistant tissues even hemorrhage
was lacking. The minimal changes that were observed in cells of the
liver and adrenal glands were indistinguishable from those seen rou-
tinely in these cells in most post-mortem studies. However, it should
be emphasized that no special stains were used and no microchemical
analyses were made to bring out elusive alterations in the parenchymal
composition of the liver and adrenal glands.

DISCUSSION

The histopathologic changes observed after total body irradiation
produce nonspecific effects similar to those brought about by any of a
number of toxic agents capable of causing widespread tissue destruction
and hemorrhage. There are no pathognomonic microscopic lesions in
animals subjected to total-body irradiation. However, if microscopic
examination reveals some degree of necrosis of the cells of the bone
marrow, spleen, lymph nodes, gonads, and gastro-intestinal tract; swell-
ing and hyalinization of collagen in connective tissue; hemorrhage and
edema in tissue spaces; or erythrophagocytosis and excess blood pigment
production and phagocytosis; and particularly, if a combination of these
lesions is observed in association with a history of exposure to total body
irradiation, a diagnosis of acute radiation injury may be made with fair
certainty. Other aids in making the diagnosis include determination of
the white blood count for evidence of reduction of lymphoid cells and

granulocytes, and examination of the plasma for a circulating anti-coagulant. Again, neither of these procedures gives pathognomonic findings, but both are diagnostic aids when occurring in company with other evidence of radiation injury.

A titratable anticoagulant has been reported in the blood of irradi-ated animals.[11-13] This, plus the fact that the blood of irradiated animals is incoagulable in a test tube,[12] poses an interesting paradox when the presence of clotted blood and fibrin in the tissues of these same animals is noted. The circulating heparin-like substance, the prolonged coagulation time of whole blood *in vitro,* and the ability to form fibrin *in vivo* do exist side by side, but the explanation is not understood.
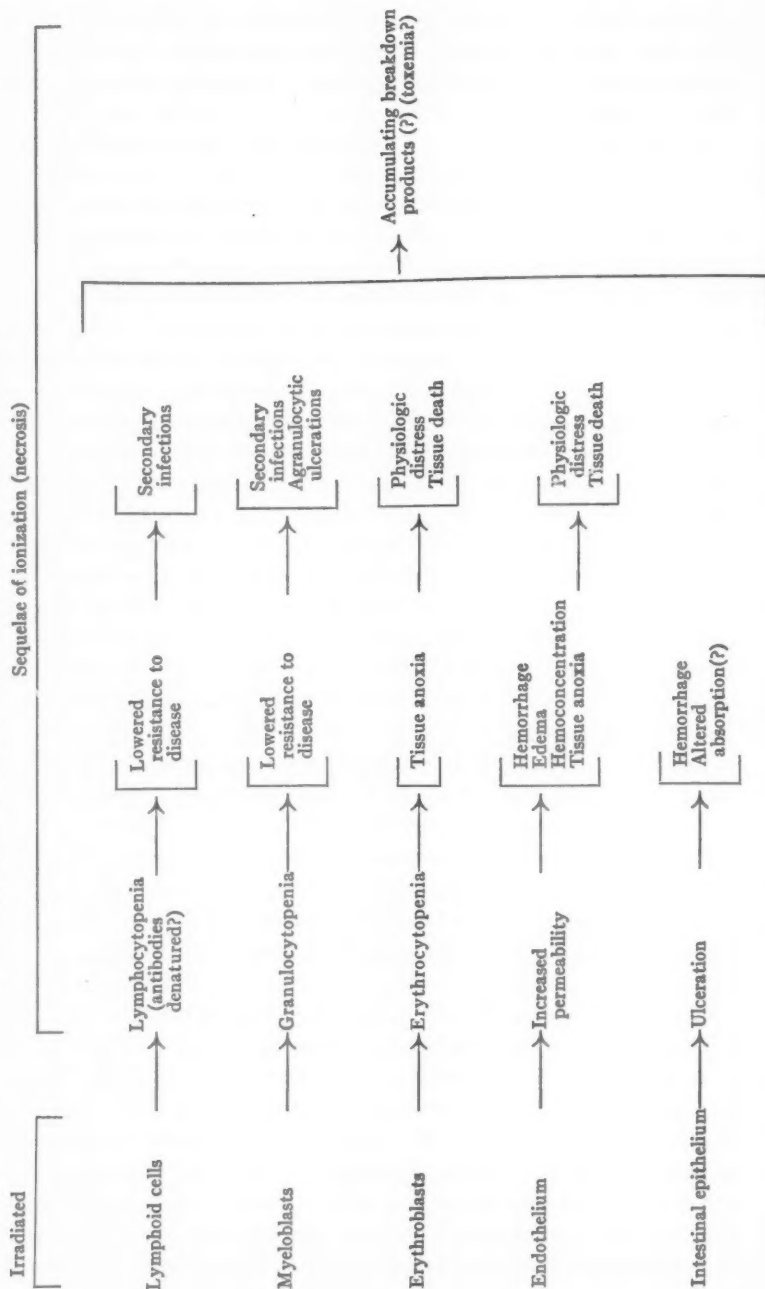
The sequence of events following total body irradiation is apparent from Table I. By destruction or injury of the hematopoietic cells of the lymph nodes, spleen, and bone marrow, the subject's resistance to infection is lowered. The cellular defense is markedly reduced and, if destruction of the lymphoid cells denatures the antibodies they carry, the humoral defense is probably reduced also.

The radiosensitive intestinal epithelium is sloughed off, leaving an ulcerated bleeding surface. If the barrier that normally prevents absorp-tion of toxic intestinal contents is injured, then it would seem probable that toxic products would be absorbed. Against this theory, however, is the normal appearance of the liver cells, which might be expected to show some evidence of toxic necrosis if toxins were being absorbed via the intestinal tract.

The widespread hemorrhage into the tissue spaces and the edema are probably the result primarily of increased capillary permeability and secondarily of changes in the circulating blood. Dilatation of capillaries is an early and generalized reaction to radiation. Dilatation, congestion, hemostasis, increased capillary permeability, and tissue anoxia follow in logical order, much as in the pattern of reaction to shock.[14] Once tissue anoxia is introduced, the "vicious cycle" becomes more and more difficult to break.

It may be that the life of the heavily irradiated subject depends on the prevention of the physiologic sequences leading to tissue anoxia. Certainly, anemia and impaired circulation with resulting stagnant anoxia must play an important part in recovery or death. Anoxia and lowered resistance to infection comprise a very formidable barrier for the injured host to overcome. To these two basic sequelae of radiation injury must be added a possible third. The very nature of the injury which produces widespread necrosis suggests that there must be an accumulation of products of the breaking down of tissues. Perhaps this

TABLE I

The Biologic Effects of Acute Radiation Injury with Reference to Selected Cells

Sequelae of ionization (necrosis)

Irradiated

Lymphoid cells ⟶ Lymphocytopenia (antibodies denatured?) ⟶ Lowered resistance to disease ⟶ Secondary infections

Myeloblasts ⟶ Granulocytopenia ⟶ Lowered resistance to disease ⟶ Secondary infections, Agranulocytic ulcerations

Erythroblasts ⟶ Erythrocytopenia ⟶ Tissue anoxia ⟶ Physiologic distress, Tissue death

Endothelium ⟶ Increased permeability ⟶ Hemorrhage, Edema, Hemoconcentration, Tissue anoxia ⟶ Physiologic distress, Tissue death

Intestinal epithelium ⟶ Ulceration ⟶ Hemorrhage, Altered absorption(?)

⟶ Accumulating breakdown products (?) (toxemia?)

produces a toxemia which either alone or in combination with the other two factors is responsible for radiation sickness and death. However, toxemia has not been proved chemically and the fairly normal appearance of liver and kidney parenchyma do not support the supposition.

Many assumptions must be clarified before radiation disease will be understood. There is one hopeful note that comes from these studies and that is that the reticular cells of lymph nodes and bone marrow are relatively radioresistant. As long as these building blocks remain intact it does not seem insurmountable to restore impaired circulation and bolster resistance to infection until such time as the host is again able to take over these functions without outside aid. The very severely damaged marrow will not regenerate, but in subjects with sublethal injury, provided they do not die from secondary causes, the possibility of regeneration of the bone marrow and recovery exists.

SUMMARY AND CONCLUSIONS

The lesions produced in swine by exposure of the total body to ionizing radiations from an atomic bomb explosion are indistinguishable from lesions produced by exposure of the total body to million volt x-irradiation. These lesions are characterized by hemorrhage, necrosis, and secondary infections.

Lymphoid cells, myeloblasts, erythroblasts, germ cells, and intestinal epithelium are found to be particularly radiosensitive. Injury to these cells causes anemia and lowering of resistance to infection.

Irradiation causes dilatation of capillaries, impairment of circulation, and tissue anoxia. Anemia enhances both lowered resistance to infection and anoxia, and thus the pathologic state becomes self-perpetuating.

Absorption of toxic substances through injured intestinal mucosa and the accumulation of products of tissue destruction in the blood stream are phenomena which logically might follow the widespread necrosis that occurs after total body irradiation. There is, however, no chemical proof or histologic evidence of toxemia from either of these sources.

Since the most primitive hematopoietic stem cells—the reticular cells —are relatively radioresistant, the effort to reduce mortality does not seem altogether hopeless. Management of total body radiation disease should be directed at prevention of secondary infections and treatment of anemia, impaired circulation, and anoxia.

REFERENCES

1. Warren, S. The pathologic effects of an instantaneous dose of radiation. *Cancer Research*, 1946, **6**, 449–453.
2. Jacobson, L. O., and Marks, E. K. The hematological effects of ionizing radiations in the tolerance range. *Radiology*, 1947, **49**, 286–298.
3. Prosser, C. L., Painter, E. E., Lisco, H., Brues, A. M., Jacobson, L. O., and Swift, M. N. The clinical sequence of physiological effects of ionizing radiation in animals. *Radiology*, 1947, **49**, 299–313.
4. Bloom, W. Histological changes following radiation exposures. *Radiology*, 1947, **49**, 344–348.
5. Desjardins, A. U. Radiosensitiveness of cells and tissues, and some medical implications. *Arch. Surg.*, 1932, **25**, 926–942.
6. Warren, S. The histopathology of radiation lesions. *Physiol. Rev.*, 1944, **24**, 225–238.
7. Draeger, R. H., and Warren, S. Medicine at the crossroads. *U.S. Nav. M. Bull.*, 1947, **47**, 219–225.
8. Tullis, J. L., and Warren, S. Gross autopsy observations in the animals exposed at Bikini. A preliminary report. *J. A. M. A.*, 1947, **134**, 1155–1158.
9. Tullis, J. L., Tessmer, C. F., Cronkite, E. P., and Chambers, F. W., Jr. The lethal dose of total-body x-ray irradiation in swine. *Radiology*, 1949, **52**, 396–400.
10. Tessmer, C. F., and Tullis, J. L. The gross and histopathologic changes in swine exposed to 1000 kv. x-irradiation. Naval Medical Research Institute, Project NM 007 039. (In preparation.)
11. Allen, J. G., and Jacobson, L. O. Hyperheparinemia: cause of the hemorrhagic syndrome associated with total body exposure to ionizing radiation. *Science*, 1947, **105**, 388–389.
12. Cronkite, E. P. The hematology of the Bikini animals. (Abstract.) *Am. J. Path.*, 1947, **23**, 891.
13. Allen, J. G., Sanderson, M., Milham, M., Kirschon, A., and Jacobson, L. O. Heparinemia (?). An anticoagulant in the blood of dogs with hemorrhagic tendency after total body exposure to roentgen rays. *J. Exper. Med.*, 1948, **87**, 71–86.
14. Moon, V. H. Hemoconcentration as related to shock. *Am. J. Clin. Path.*, 1941, **11**, 361–387.

DESCRIPTION OF PLATES

PLATE 115

Figures 1, 3, and 5 are photographs of lesions in swine exposed to ionizing radiations from the atomic bomb, while Figures 2, 4, and 6 are counterparts in animals exposed to million volt x-irradiation.

FIG. 1. Extensive purpura of the skin. Bikini pig 330, 13 days after irradiation.

FIG. 2. Similar to Figure 1. Local pig F, 16 days after 600 r. delivered bilaterally, measured in air.

FIG. 3. Hemorrhage and edema in the oral pharynx with ulcers (arrows) in the tonsillar tissue. Bikini pig 402, 13 days after irradiation.

FIG. 4. Similar to Figure 3. Local pig 470, 13 days after 400 r. delivered bilaterally, measured in air.

FIG. 5. Hemorrhage in the myocardium of auricles and ventricles. Bikini pig 288, 11 days after irradiation.

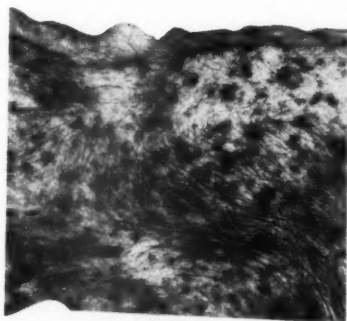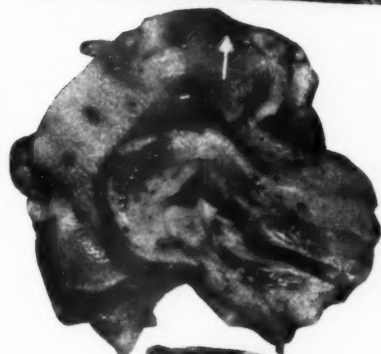FIG. 6. Similar to Figure 5. Local pig E, 16 days after 600 r. delivered bilaterally, measured in air.

Tullis

Total Body Irradiation

PLATE 116

Figures 7, 9, and 11 are photographs of lesions in swine exposed to ionizing radiations from the atomic bomb, while Figures 8, 10, and 12 are counterparts in animals exposed to million volt x-irradiation.

FIG. 7. Multiple ulcers in the stomach and numerous petechial hemorrhages in the mucosa of the stomach and duodenum. Bikini pig 240, 8 days after irradiation.

FIG. 8. Similar to Figure 7. Local pig 418, 15 days after 400 r. delivered bilaterally, measured in air.

FIG. 9. Multiple hemorrhagic areas in the bowel, visible through the serosa. The mesenteric lymph nodes are enlarged, edematous, and hemorrhagic. Bikini pig 393, 11 days after irradiation.

FIG. 10. Similar to Figure 9. Local pig A, 14 days after 400 r. delivered bilaterally, measured in air.

FIG. 11. Massive hemorrhage in the pelvis of the kidneys (Bikini pig 240) 8 days after irradiation, and hemorrhages in the testicle (Bikini pig 456) 13 days after irradiation.

FIG. 12. Similar to Figure 11. Kidney from local pig XU and testicle from local pig U, 28 days after 600 r. delivered bilaterally, measured in air.
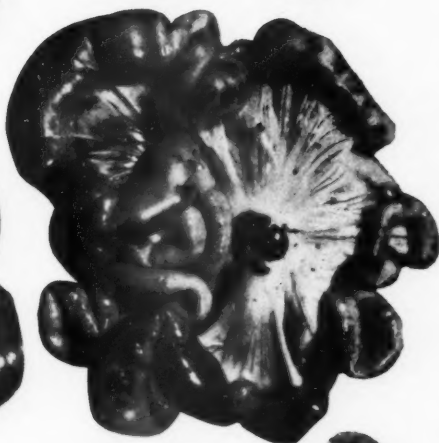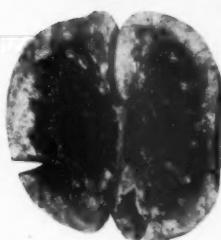
Tullis

Total Body Irradiation

PLATE 117

All photomicrographs—Figures 13 through 18—are taken from lesions in swine exposed to ionizing radiations from the atomic bomb tests at Bikini.

FIG. 13. Bone marrow of pig 240, 8 days after irradiation. Of note is the general washed-out appearance of the marrow and absence of hematopoiesis. × 200.

FIG. 14. Lymph node of pig 459, 5 days after irradiation. There is diffuse hemorrhage and marked reduction in lymphoid elements. × 100.

FIG. 15. Bone marrow of pig 240. In a magnified portion of Figure 13 there are five surviving, normal-appearing reticular cells and one unidentified white blood cell (myelocyte?) in addition to scattered erythrocytes. × 600.

FIG. 16. Lymph node of pig 265, 6 days after irradiation. The lymphoid elements are depleted, causing relative prominence of reticular cells which are unharmed by irradiation. Thickening and hyaline change in the reticulum network of the nodule may be noted. A small vessel near the nodule is occluded with an agglutinative thrombus. × 200.

FIG. 17. Erythrophagocytosis, pig 374. Seven days after irradiation a free macrophage is in the sinus of a lymph node. There are five or six ingested red blood cells and two dark, irregular masses in the cell. The latter are ingested blood pigment granules. The nucleus is not shown. × 900.

FIG. 18. Lymph node of pig 240, 8 days after irradiation. There has been considerable hemorrhage and destruction of blood, with scattered deposition of blood pigment granules both within and outside phagocytes. × 200.
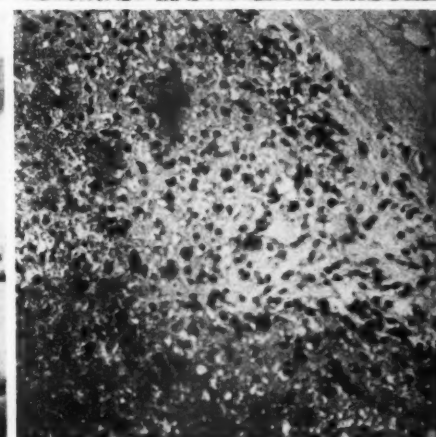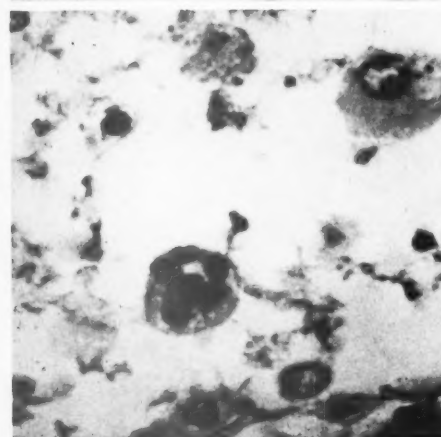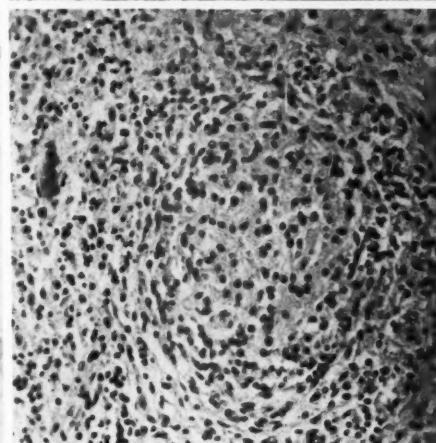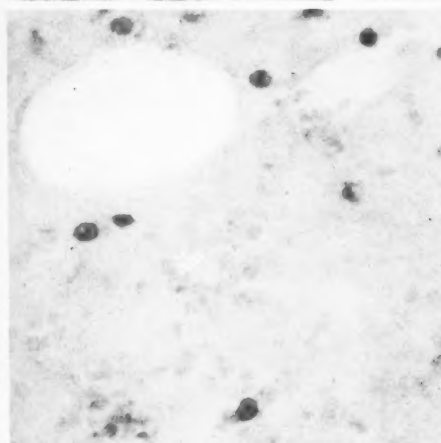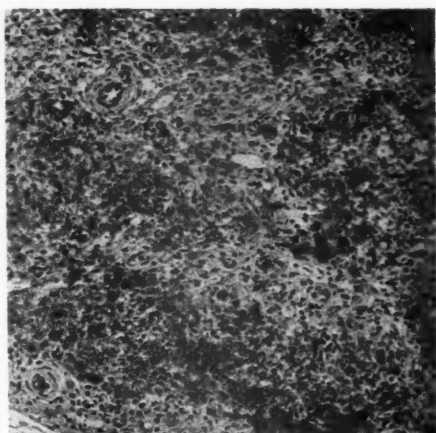
Tullis

Total Body Irradiation

All photomicrographs—Figures 19 through 24—are taken from lesions in swine exposed to ionizing radiations from the atomic bomb tests at Bikini.

FIG. 19. Lymph node of pig 265, 6 days after irradiation. The wall of the dilated sinus is undergoing necrosis of a coagulative type with a deposition of fibrin on the surface. The vessel on the right margin of the field is occluded. × 200.

FIG. 20. Lymph node of pig 265. Higher magnification of a portion of the node in Figure 19. Detail of the fibrin deposit on the necrotic tissue is brought out. In addition, it should be noted that many of the surviving cells in the node are of the reticular type. × 400.

FIG. 21. Spleen of pig 459, 5 days after irradiation. There is a marked reduction in the lymphoid elements in the region of the malpighian corpuscles. × 200.

FIG. 22. Tonsil of pig 459, 5 days after irradiation. The vessels are dilated and congested. Several of them appear completely occluded with agglutinated erythrocytes. The lymphoid elements in the stroma of the tonsillar tissue are markedly depleted. × 100.

FIG. 23. Necrosis in a follicle of a tonsil of pig 240. Of note is the lack of cellular response to the necrosis. × 200.

FIG. 24. Necrosis and ulceration in the mucosa of the large intestine of pig 459. There is not only a lack of cellular response to necrosis but the lymphoid elements normally seen in the lamina propria are depleted. Also the fibrin network covering the ulceration is nearly devoid of cells. × 100.
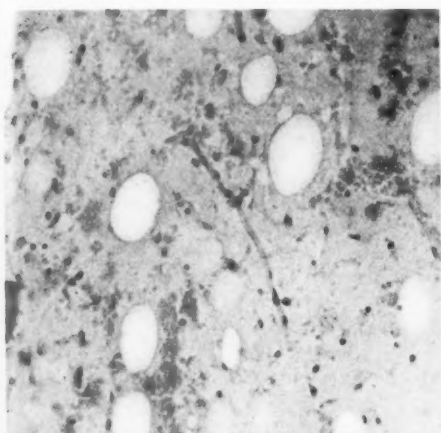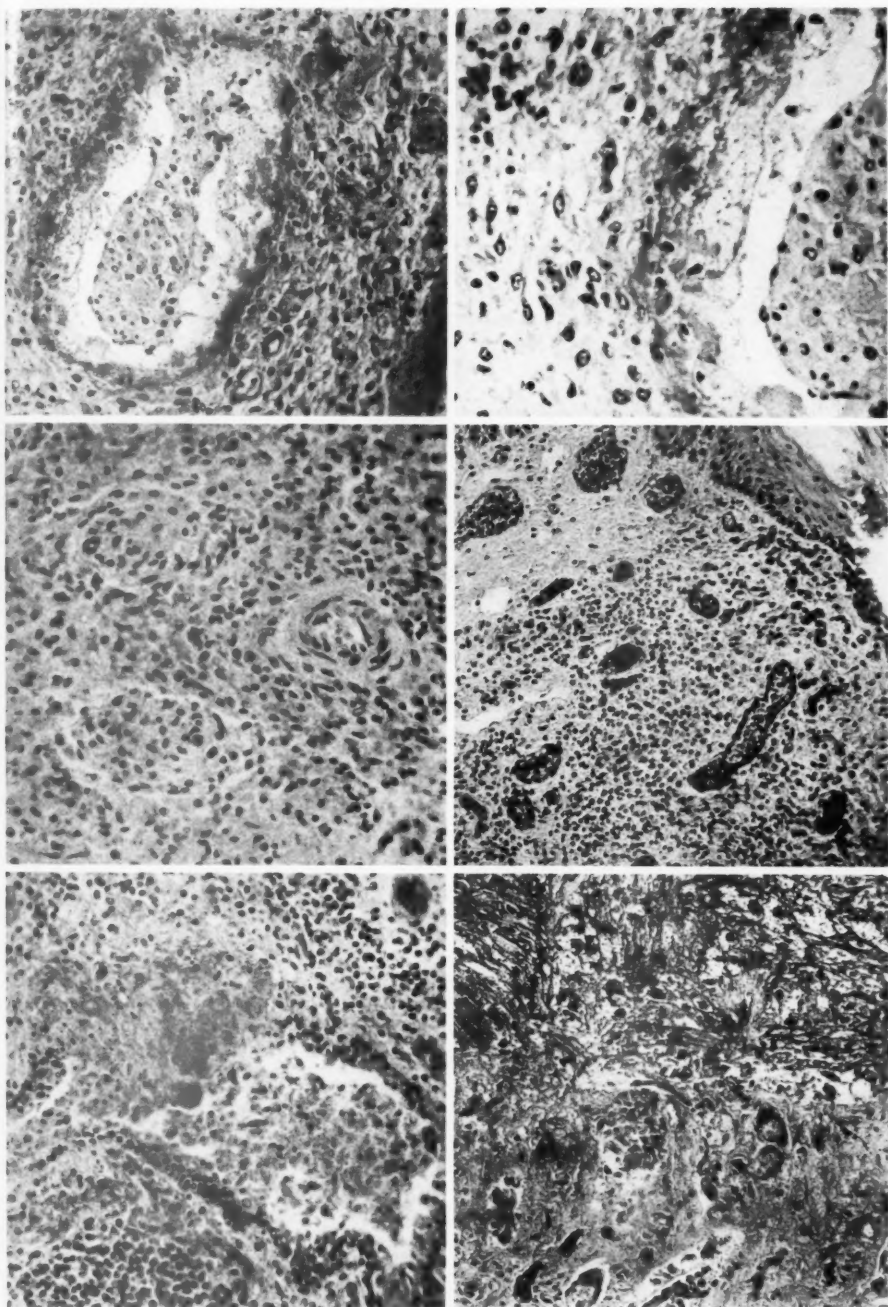
PLATE 118

Tullis

Total Body Irradiation

All photomicrographs—Figures 25 through 30—are taken from lesions in swine exposed to ionizing radiations from the atomic bomb tests at Bikini.

FIG. 25. Small intestine of pig 419. This is at the junction of an ulcer and a less involved portion of mucosa. There is a sharp line of demarcation between the totally necrotic area and the area in which there is distortion, vacuolation, and pyknosis in the epithelium. × 200.

FIG. 26. Small intestine of pig 419. Fibrin thrombi and one agglutinative thrombus in vessels beneath the mucosa and ulcerated area seen in a portion of Figure 25. × 200.

FIG. 27. Fibrillar degeneration of connective tissue cells in the loose areolar tissue of the subserosa of the duodenum of pig 240. There is some extravasated blood and edema fluid. Degeneration of this type was seen in connective tissue elements in other parts of the body. × 400.

FIG. 28. Stomach mucosa of pig 419. There is marked variation in the cells making up the glands. Some are vacuolated, some pyknotic and some assume bizzare, distorted forms. × 400.

FIG. 29. Lungs of pig 337. Edema, fibrin, and scattered macrophages are found in the alveoli 12 days after irradiation. The vessels of the alveolar septa are congested. There is no inflammatory cellular infiltration. × 300.

FIG. 30. Lungs of pig 337. Hemorrhage in the alveoli and an agglutinative thrombus in an arteriole are seen in a different portion of the same lung shown in Figure 29. × 300.
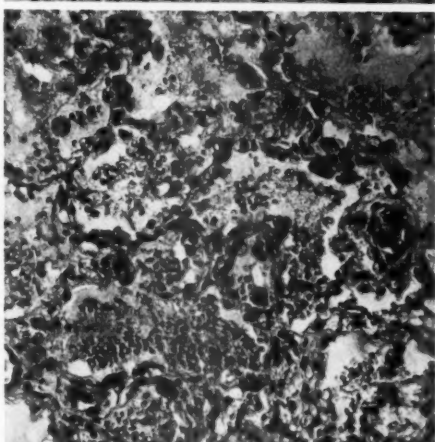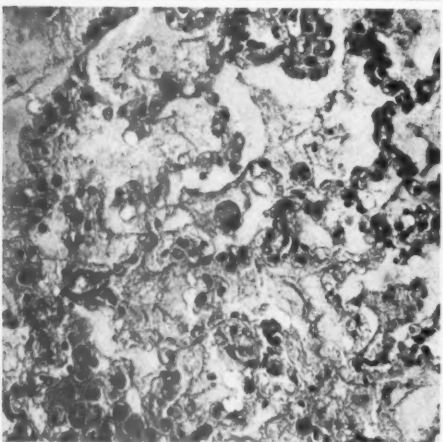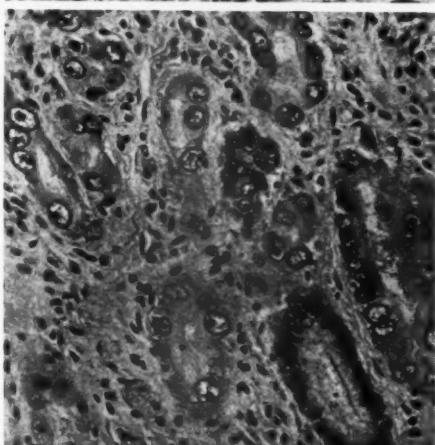
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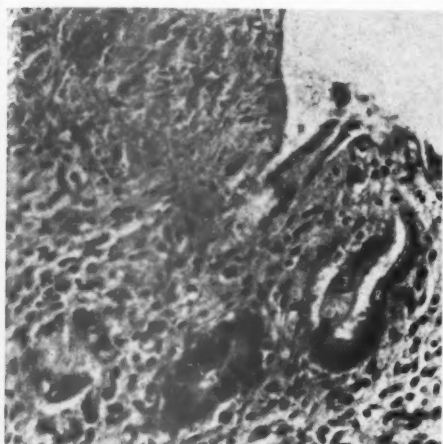
Tullis

Total Body Irradiation

PLATE 120

All photomicrographs—Figures 31 through 36—are taken from lesions in swine exposed to ionizing radiations from the atomic bomb tests at Bikini.

FIG. 31. Kidney of pig 419. Small necrotic focus in tubular epithelium associated with hemorrhage of interlobular vessels. The surrounding tubules and two glomeruli appear normal. × 100.

FIG. 32. Bladder of pig 364, 7 days after irradiation. The mucosal epithelium is degenerated and most of it has been sloughed off. The subepithelial tissue is edematous and one vessel is occluded with an agglutinative thrombus. × 300.

FIG. 33. Testis of an immature boar, pig 459, 5 days after irradiation. The spermatogonia are nearly all destroyed. The ghost outline of one or two of them may still be seen. The seminiferous epithelium is reduced to one cell layer composed chiefly of so-called indifferent cells. × 400.

FIG. 34. Ovary of a sow, pig 364, approximately the same age as the boar shown in Figure 33, 7 days after irradiation. Most of the residual ova are atretic while others are vacuolated and appear to be degenerating. The stroma is edematous. × 300.

FIG. 35. Skin of ear of pig 265. Vacuolation of nuclei and clumping of chromatin are found in the epidermis, chiefly in the basal layer, 6 days after irradiation. The dermis is edematous and one vessel contains an agglutinative thrombus. × 400.

FIG. 36. Hair follicle of pig 265. There is no clear definition of the several epithelial layers that envelop the root of the hair and many of the cells are vacuolated. × 200.

PLATE 120
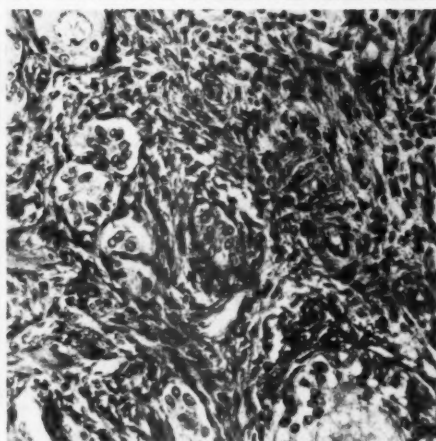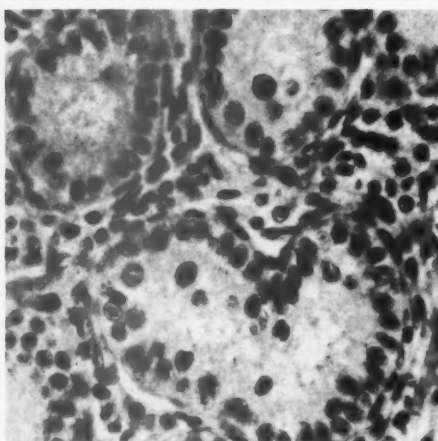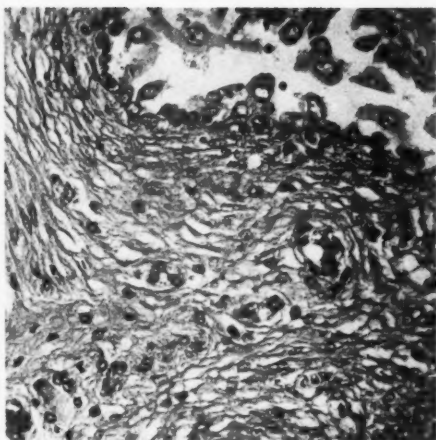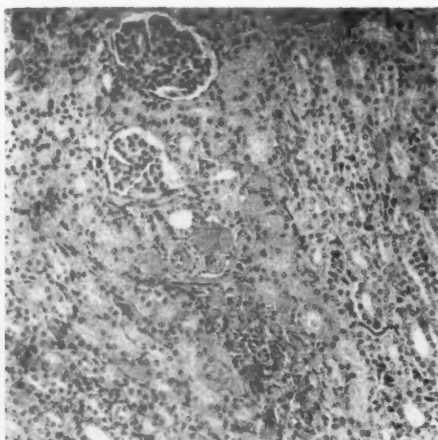
Tullis

Total Body Irradiation

PATHOLOGY OF ATOMIC BOMB CASUALTIES*

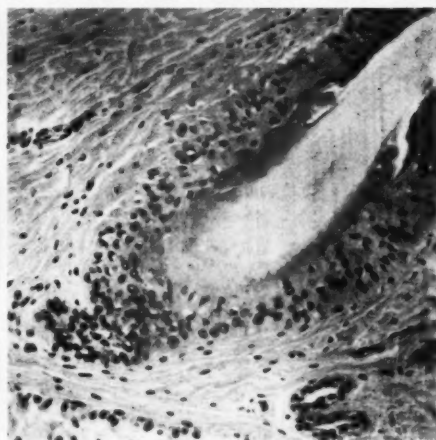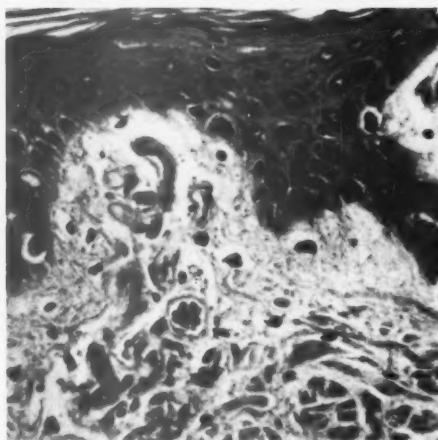AVERILL A. LIEBOW, M.D. (Department of Pathology, Yale University School of Medicine,
New Haven, Conn.), SHIELDS WARREN, M.D. (Department of Pathology, Harvard
Medical School and New England Deaconess Hospital, Boston, Mass.),
and ELBERT DeCOURSEY, Col., M.C., U.S.A. (Army Medical Depart-
ment Research and Graduate School, Washington, D.C.)

*(From the Army Institute of Pathology, Atomic Energy Commission, Naval Medical
Research Institute, and Department of Pathology, Yale University
School of Medicine)*

The energies released in atomic explosions embrace the entire range
from that of mechanical shock waves to that of gamma rays. The effects
on exposed tissues are modified not only by the characteristics of the
bomb but by the conditions of exposure. As it happened, the injuries
were not qualitatively different in Hiroshima and Nagasaki. The descrip-
tions of the lesions must, however, be regarded as representative only
of the changes produced at those two cities. Whether or not the explosion
of other bombs, or of like bombs under different conditions, would pro-
duce comparable effects cannot be stated.

METHODS AND MATERIALS

Since the Joint Commission was not able to begin its work in Japan
until some 6 weeks after the bombings of August 6, 1945, at Hiroshima
and August 9, 1945, at Nagasaki, by which time most of those who were
to die from radiation effects had succumbed, it was necessary to secure
clinical records and necropsy protocols and specimens from Japanese
clinicians and pathologists to supplement materials obtained at necropsy
by members of the Joint Commission itself. Some of these pathologists
were attached to the Commission in a group of some sixty Japanese
physicians who were to aid in the clinical field study. Others had been
with "research parties" that had been sent into the stricken cities by
various universities and by the Japanese Army Medical Service to
conduct medical investigations. To the last mentioned we owe the only
available records and materials from the patients dying within the first

few days following the bombing. These were obtained for study through
the aid of the Japanese Surgeon General Hiraye. Many of these "research
parties" were staffed by the best available Japanese scientists who did
work of a high order of excellence. Gradually a list of these pathologists
was constructed—no small task since provincialism and professional
jealousy were not unknown in the academic circles of Japan. In the
work of establishing contact with these men and obtaining the necessary
materials, the Joint Commission was greatly aided by Dr. Masao
Tsuzuki, then Professor of Surgery at the Tokyo Imperial University,
and chief of the medical section of the Japanese National Research
Council. It was his influence that unlocked cabinets of material that
could otherwise easily have remained sealed. The records were trans-
lated word by word from the original protocols by a team comprised of
the pathologist who had performed the necropsy, one of the English
speaking pathologists attached to the Joint Commission, and one of the
American pathologists of the Commission. In many instances it was
necessary to travel into remote parts of Japan to obtain the records and
materials at first hand from the files of the smaller universities. When
gross specimens were still available, they were checked against the
descriptions in the protocols. Most of these were found to be quite
accurate. Blocks were then taken from the original specimens to check
and supplement the usually better fixed tissue in the smaller specimen
jars and also the paraffin blocks and finished histologic preparations that
were requested in the original letters written by Dr. Tsuzuki. The histo-
logic slides were checked also against the protocols as they were received.

The records and materials were brought back to the United States
where additional sections were prepared and illustrations made through
the facilities of the Army Institute of Pathology. The Naval Medical
Research Institute aided materially in working up the Nagasaki material.
The necropsy protocols were transcribed into standard United States
Army Medical Department form. Each was assigned a "Key Number,"
by which it is filed under the general A.I.P. accession number 158930.
Reference to patients in the present text is made according to this key
as "K" followed by the appropriate numeral.

After a preliminary review, tissues from 170 patients were found
sufficiently well preserved to warrant detailed histologic study. For pur-
poses of statistical tabulation, however, only the last 16 necropsies of
the Nagasaki series, which had been performed after the 40th day by
members of the Joint Commission itself, were found useful, since only in
those protocols was there a record of the distance from the point of

explosion, and of possible protective factors. These factors usually were recorded in the Hiroshima protocols since the Japanese pathologists there had been keenly aware of their supreme significance. Moreover, many of the patients had been soldiers within well defined areas of the large military encampment (Chugoku Army Headquarters), one of whose boundaries was almost directly beneath the point of explosion of the bomb. Consequently, the detailed tabulations are based on a total of 110 patients, 94 from Hiroshima and 16 from Nagasaki, although occasional reference is made to the earlier Nagasaki material. It may be unequivocally stated that at least the temporal sequence of anatomic changes in heavily exposed patients in the two cities is similar. Lamentably, an exact comparison by distance from the bomb is not possible on the basis of the material at hand.

The concurrent clinical studies of some 14,000 patients, and the protection and casualty surveys are summarized in the general Medical Report of the Joint Commission.[47]

THE INJURIOUS FACTORS—GENERAL STATEMENT

The physical factors of distance and the condition of the blanket of atmosphere at the moment of explosion are two important determinants of the effects of the bombs. Both bombs were exploded many hundreds of yards above the cities. In the discussion to follow the distances are stated from the point on the ground above which the explosion occurred, not from the explosion itself. The former point is variously referred to as the "hypocenter," "ground center," or "ground zero."

The injurious factors and the damage inflicted are summarized in

TABLE I

Energies and Effects of the Atomic Bomb

Energy	Injury	Type or target of injury
I. Mechanical	A. Trauma	1. Direct (blast)
		2. Indirect (falling débris)
II. Radiant		
a. Thermal	B. Burns	1. Direct ("flash burns")
		2. Indirect
b. Ionizing radiations	C. Radiation effect	1. Skin
		2. Gastro-intestinal tract
		3. Gonads
		4. Lymphoid tissue
		5. Marrow
		6. Other tissues

general terms in Table I. Often the three main types of injury coexisted: traumatic, thermal, and ionizing radiation. For purposes of clarity, these will, however, be discussed separately. Traumatic and thermal injury accounted for the vast majority of the casualties.

Traumatic Injuries

In considering the mechanical effects of the explosion, direct blast injury and the indirect effects produced by collapsing buildings and flying débris must be distinguished. As in the bombings of Great Britain, the latter was far more important in producing casualties than the former.

Direct blast injury to ears, lung, and intestines analogous to that inflicted by high explosive bursting within a few feet was almost unknown. At the prevailing distance from the target the explosion did not have the hammer-blow effect of high explosive,[20,98] but rather was like a sudden violent gust of air which lasted for a brief but appreciable period. There was a short, positive phase followed by a longer, but less destructive, negative phase. The relative ineffectiveness of the latter was indicated by the nature of the damage to buildings, most of which showed a preponderance of effects indicating a thrust away from the bomb. At a Hiroshima hospital soon after the bombing only 8 of 371 patients who were examined had ruptured ear drums, although 19 suffered temporary deafness. Seventy-six per cent of this group of men had been within 2000 yds. of the center. In the survivors examined by the Joint Commission after the sixth week, less than 1 per cent, even of those who had been within the first 1000 yds., had ruptured ear drums, and beyond 1500 yds. the incidence fell below 0.1 per cent. Not a single middle ear or tympanum was available for microscopic examination from the patients in whom there was a clinical suggestion of blast injury. The foci of pulmonary emphysema and atelectasis without hemorrhage observed in some of the early casualties (Fig. 20) are difficult to interpret. These were found frequently at death in patients who had not been exposed to blast. Many patients lost consciousness for brief periods, although they could not remember any direct trauma aside from blast. A few were injured by being hurled forcibly, sometimes for considerable distances, against solid objects, but this must be considered an indirect effect of the blast.

Immensely more injury was inflicted by the indirect effect of the blast —the falling débris and masonry. How many were killed outright will never be known accurately. Thousands were pinned beneath the wreckage and were soon consumed by the holocaust of fire that swept the city and which made rescue impossible. In October of 1945, 2 months after the explosion, it was remarkable to note how few were the survivors who had suffered really severe injury. Thus in survivors at Hiroshima the incidence of fractures was less than 4.5 per cent. It was not that injuries

were few; rather, almost none who had lost the capacity to move escaped the flames.

Very characteristic were the lesions inflicted by flying glass (Fig. 1). This material often split into long spear-shaped fragments, even as far as 3 miles from the explosion. The fragments were hurled with such force as to become embedded in the wood of the opposite walls. As was to be expected, the wounds were multiple and often penetrating. Often keloids appeared following injury of this type (as is not infrequent in the Japanese race), and arteriovenous aneurysm sometimes resulted from injuries of the vessels.

Since some of the patients had suffered injury also from ionizing radiations with consequent leukopenia, it is obvious that even minor traumatic wounds might become the seat of infections which could become generalized.

Burns

As in the case of the mechanical forces, direct and indirect factors in the causation of the burns must be considered. Direct "flash" burns resulted from exposure to the radiant energy of the bomb. This energy was transmitted in a spectrum resembling that of the sun. In the present section only that band including the ultraviolet, visible light, and infra-red rays will be considered. The exact intensities of these various components cannot be stated, but it is probable that all contributed to the damage to the skin of exposed human beings. All of the component rays were transmitted in straight lines at the velocity of light. Consequently, only surfaces directly exposed were burned and intervening objects cast "shadows."

To understand the "flash burns" one must conceive of a very large amount of radiant energy acting for an extremely brief interval of time. The intensity is indicated by the effect on granite which within several hundred yards showed fragmentation of its surface caused by an unequal expansion of its components. It has been estimated that a temperature of at least 2000° C. is necessary to produce this effect. The brevity of the peak intensity of the flash is indicated by the fact that the "shadows" of constantly moving and easily shriveled objects, such as leaves, are sharply outlined on wood behind them—and exposed wood was super-ficially carbonized even at 3000 yds. from the center.[47]

The rays in their rectilinear course burned whatever profile, including that of man, directly faced the center of the explosion. Thus the burns typically were very sharply outlined. Intervening objects also cast sharp protective shadows on the skin. Those close to the bomb suffered searing

or charring of the skin to the level of the subcutaneous tissue and died shortly (Fig. 3). Death was the fate of more than 95 per cent of those directly exposed to the bomb within 1200 yds. of the point above which the bomb exploded. Beyond this, clothes were some protection. The darker shades absorbed more heat as is demonstrated by selective scorching of the dark polka dots, or stripes, or flowers on a contrasting lighter pattern. Occasionally, such parts of the skin as underlay the darker portions of the pattern were selectively injured (Fig. 2). Sometimes, however, burns occurred beneath unaltered cloth, where it was tightly stretched over the skin in those patients closest to the explosion (Fig. 4).

Between 1500 and 2000 yds. the burns varied in their severity, some showing complete destruction of the derma, others being of second degree. From the data of Ashe and Roberts[5] it is learned that a temperature of 400° C. acting for approximately 0.5 seconds is necessary to produce a second degree burn. The exact numbers of calories absorbed by various surfaces exposed to the bomb are not known, since the duration and intensity curve of the heat flux are matters of conjecture. As 2000 yds. was approached, some cutaneous surfaces ultimately showed, after an initial phase of erythema and blistering, depigmentation with superficial damage or no damage to the epithelium (Fig. 4). Beyond this distance, and up to 4000 yds., the opposite change, an intense pigmentation resembling that of ordinary but extreme sunburn, followed the initial erythema. This deep chocolate colored mask was well shown in a group of men who had been prisoners in the city jail at approximately 2500 yds. from the hypocenter (Fig. 5).

Closer inspection of the sharply outlined "profile" or "flash" burns after healing showed that even the most severely burned areas usually were surrounded by an intensely pigmented zone, and this, in turn, by a narrow band of depigmented tissue whose overlying epithelium was intact (Fig. 8). Even the centrally depigmented but otherwise little damaged skin showed similar phenomena of pigmentation—a broad pigmented area surrounding the central, most-directly exposed zone of depigmentation, with a narrow ring of depigmented tissue again separating the hyperpigmented skin from the surrounding normal tissue.

Practically all of the burns became infected, and many which had been of the second degree ultimately showed deep destruction of tissue as a result of bacterial action. The sharp outlines of the burns often were disturbed, as undermining ulcerations of bacterial causation developed. The infections, as in the case of the mechanical injuries, were particularly

important in those who were within 1500 yds. and who had received a significant dose of radiation.

Occasionally the thermal injury, coupled with infection, resulted in chondritis of the ear, which, in healing, produced a scarred, shrunken, and distorted auricle.

Keloids were observed frequently and were extreme in some patients (Fig. 7) within 10 weeks. Their subsequent development has been described by Block and Tsuzuki.[9] More than three-fourths of the healing second and third degree burns showed some overgrowth of scar tissue. The tentative conclusion of the Japanese observers was that keloids probably were not more frequent than would be expected in this race in ordinary, infected, poorly treated burns. This conclusion has not been altered by subsequent observations of the survivors.[9]

Histologically, there is evidence confirming the gross observation that depigmentation of the skin can occur even if the epithelium of the surface is not completely destroyed (Figs. 6, 11, and 12). In that portion of the skin which had been most severely burned, the epithelium of the surface was necrotic and contained pyknotic nuclear remnants, both of polymorphonuclear cells and large mononuclear phagocytes. Very few leukocytes were present in the adjacent corium and deeper lying perivascular tissue. At the margins of the denuded area the epithelial cells of the malpighian stratum had lost their pigment and no dermal melanophores were found. Some other epithelial cells had become vacuolated and were in disarray. At a still greater distance from the zone of destruction the epithelium was relatively well preserved and its basal layers had become excessively pigmented. There some dermal melanophores also were seen. More laterally still there was a much less pigmented zone but there again was evidence of a more deeply penetrating burn. The zone of hyperpigmentation corresponded to that observed grossly. In this particular case, however, there had occurred also some severe injury more peripherally which precluded complete interpretation of the gross appearance. These observations indicate that complete destruction of epithelium is unnecessary for depigmentation to occur. They suggest also that some special band of the spectrum, probably in the ultraviolet, might be responsible for this phenomenon. The diverse effects upon the skin of different bands in the ultraviolet have been pointed out recently.[12,13] Much is still to be learned concerning the action of high intensities in the ultraviolet range.

The results of infection in the presence of leukopenia were seen in a section from a small burn in a patient who was at 1000 yds. and who received no other serious burns. The epithelium had completely sloughed

(Fig. 13). On the homogeneous, dull pink-staining surface of the collagen were present irregular, granular, purple-staining masses of cocci. Some of these occurred in islets as much as 1 mm. below the surface and one clump actually lay within a bundle of smooth muscle (arrector pili). Squamous metaplasia had occurred in the ducts of the sweat glands. There appeared to have been actual proliferation of these cells, many of which were large and irregular with tremendous vesicular nuclei. The acini of the sweat glands, however, exhibited shrinkage of the epithelium and irregular thickening and compaction of the basement membrane, in some cases with partial collapse of the acinus. Nowhere was there a leukocytic infiltration in the vicinity of the bacterial masses. In one small portion of the surface a very thin layer of squamous epithelial cells remained. These cells were much swollen, some were multinucleated, and in all instances the vesicular nuclei contained very prominent chromatin knots. These changes are ascribed to thermal effect, but what direct rôle ionizing radiations played is difficult to assess. The evidence of indirect effect of the ionizing radiations lies in the total absence of leukocytic response to the injury and to the bacteria that are seen in section.

In another case, the subepithelial tissues contained a scanty exudate consisting largely of monocytes situated within extravasated fibrinous material. One vessel was found to have a necrotic wall and a lumen filled with a thrombus which consisted largely of fibrin (Fig. 14). Deeper in the subcutaneous tissue of the same specimen was a remarkable basophilic ground substance containing irregular large cells with basophilic cytoplasm. Some of these had the granules of mast cells (Fig. 15).

Severe burns caused by contact with flame rarely were observed by the Joint Commission, for the same reason that relatively few severe traumatic injuries were seen among the survivors. The gathering fires spread so rapidly within the cities that only those who were not severely injured could escape. Occasionally, dark clothing burst into flame on those within 1500 yds. of the hypocenter and contact burns resulted. Such burns lacked the sharp outlines produced by shadowing, and the "profile" characteristics seen in the flash burns. Their course also was affected by infection and leukopenia.

Radiation Effects

Etiologic Factors

During the explosion of the atomic bomb, vast quantities of ionizing radiations were released in the form of gamma rays, neutrons, beta particles, alpha particles, radioactive fission products, and possibly some

of the unexploded radioactive substance of the bomb itself. Factors of distance and shielding are of special importance in considering the effects of these radiations. Thus, the beta and alpha particles can be disregarded since they travel only a short distance through air. The fission products largely were swept into the stratosphere by the violent updraft created by the heat of the explosion. There was no biologic evidence that significant amounts of these materials had been deposited upon the target. Potentially of greater significance may be the neutrons, for they can be projected for considerable distances through the atmosphere. Their effectiveness in damaging tissue is several times greater than that of gamma rays, measured in roentgens equivalent physical (rep).[41] The complexity of their interaction with tissue has been described by Zirkle.[97] Furthermore, they can induce radioactivity in various materials. While there is no evidence of a significant amount of induced radioactivity at the hypocenter (as attested by the absence of radiation effects in the Ishizuka military unit which had been sent to this area for rescue work after the explosion[47]), nevertheless, neutrons in their passage through the atmosphere produce a certain proportion of the highly penetrating gamma radiations. Since at any point there was a complex of radiations, the inclusive term "ionizing radiations" will be employed hereafter. There is justification for this in that no qualitatively different effects have been observed in the tissues upon exposure to the various types of radiations.[10,79,90] In each instance the damage inflicted is proportional to the quantity of energy absorbed, not to the quantity delivered.[90] It follows that the direct injury is localized to the sites where ionization occurs.[70]

The gamma rays merit special discussion, since they were probably by far the most important of the biologically damaging radiations emitted from the bomb at the instant of detonation and from the rapidly rising column of fission products. The higher the energy and the shorter the wavelength the greater is the penetrating power in tissue. Radiations of longer wavelength ("soft" gamma rays) tend to be absorbed in the superficial tissues, while some of those of shorter wavelengths may pass entirely through the body with little or no absorption. Thus the wavelength mixture as well as the distance from the explosion will determine the localization and extent of the greatest damage. During the explosion of the atomic bomb a spectrum of gamma rays of widely varying wavelengths was emitted. The proportions of the gamma ray energy of these various wavelengths in the radiations in Japan are not known. Moreover, the gamma rays are to some degree scattered by intervening materials, including air, thereby modifying the wavelength by the Comp-

ton effect,[87] with the result that very little is known about the quality of the radiations actually delivered to the body. Another factor of biologic importance is the dose rate. Within limits still to be determined, the more rapidly a given quantity of radiation is administered, the greater its injurious effect.[21,41] In the case of the atomic bomb the total duration of exposure was, at most, a few seconds, and probably by far the greatest dosage was delivered in a very small fraction of a second.

Although the exact nature, spectrum, and intensity of the ionizing radiations produced by the atomic bomb are incompletely known, their action upon the tissues is analogous to that of ordinary x-rays. The most striking changes were found in the skin, gastro-intestinal tract, testes, lymphatic system, and bone marrow. As a consequence of the direct damage to marrow, there resulted all of the tissue changes characteristic of aplastic anemia and of the associated infection. Thus, again, direct and indirect effects were manifest.

As to the range of the radiation effects, it may be stated that severe evidences of radiation injury were rare beyond 1500 yds. from the hypocenter, if they existed at all.

Clinical Classification

Patients with radiation injury fall into four major groups which will receive brief discussion. The clinical observations are presented in detail in the body of the Medical Report.[47]

Group I: Patients dying in the first and second weeks.

Group II: Patients suffering severe symptoms or dying in the third, fourth, fifth, and sixth weeks.

Group III: Patients dying after the sixth week.

Group IV: Mild cases.

Group I. The factors responsible for death during the first 2 weeks may be: (1) An unusually high dosage of ionizing radiations either because of proximity to the bomb, or because of lack of efficient shielding. (2) An unusual susceptibility to ionizing radiations. Individual as well as species variations in susceptibility have often been observed.[38] (3) Concomitant severe thermal or traumatic injuries, or intercurrent infections, or causes of death not directly connected with the bombing. Multiple injuries were the rule.

With the exception of this last sub-group, which will be ignored for the present, many patients suffered during the first few days from a fatal illness that to the Japanese medical observers at first seemed mysterious,

until it was realized that they were dealing with the syndrome of "radiation sickness." The signs and symptoms in general resembled those found in animals after exposure to massive doses of ionizing radiations,[60] and probably represented largely the direct effects of these radiations, complicated in some instances by infection.

Patients complained of nausea and vomiting, often within $\frac{1}{2}$ hour of the bombing, or during the succeeding hours or days. This was followed shortly by anorexia, malaise, severe diarrhea that in a few instances became sanguineous, intense thirst, and fever that ascended step-wise day by day. Death ensued in coma or delirium within the first 2 weeks. Leukopenia, especially lymphopenia, was found as early as the first day after the bombing in some of these patients, but others had no leukopenia at the time of death as late as 7 days after the bombing. Thrombocytopenia and an increasingly severe anemia appeared in that order, but usually not until after the second week.

In this early group there was already histologic evidence of radiation effect upon the hair follicles, gastro-intestinal tract, lymphoid tissues, bone marrow, and gonads, but neither epilation nor purpura had become clinically manifest in the vast majority of patients.

Group II (Patients Dying During the Third, Fourth, Fifth, and Sixth Weeks, or Surviving Severe Clinical Symptoms). In group II there were not only the clinical evidences of the direct damage by ionizing radiations such as epilation, but also the manifestations of aplastic anemia consequent upon destruction of the bone marrow. Bacterial infection, an indirect result of the radiation, was, as usual, responsible for the necrotizing lesions characteristic of the aplastic anemia. Purpura was now a frequent sign. Several factors were probably concerned in the pathogenesis of this condition: at first, probably the appearance in the blood of a substance with the properties of heparin[4]; later, thrombocytopenia which often reached levels below 10,000 per cmm.; and possibly also infection and vitamin deficiency with their effect on capillary fragility.

The usual sequence of events was as follows: Nausea and vomiting on the day of the bombing were the first evidences of the disease, followed by any or all of the symptoms characteristic of group I, but in milder form and persisting for only a few days. There was then a complete remission, until the appearance of epilation some 2 weeks after the bombing. Approximately 5 days after this, accompanied by increasing malaise, there was a daily ascending unremittent fever. At approximately the same time pharyngeal pain might appear or it came somewhat later. Petechiae and ulcerative lesions of the skin, lips, mouth, and pharynx became manifest within a few days after onset of the febrile episode.

Sanguineous diarrhea, associated with an ulcerative gastritis and enteritis, was frequently a prominent symptom at the height of the disease and sometimes appeared very early, as previously described. Leukocytes and platelets reached very low levels at the time of the fever and there was often severe anemia. The patient usually died within 1 or 2 weeks of the onset of the fever, approximately 1 month after the bombing.

Some patients exhibiting all or several of the symptoms of purpura, gingivitis, and severe or even necrotizing pharyngitis survived after a febrile period. Pharyngitis ceased before, petechiae before or during, and oropharyngeal lesions usually after the end of the febrile period. Recovery was associated with an increase in the circulating leukocytes and platelets. Many of these patients remained in an anemic and generally debilitated condition for long periods. In survivors the red blood cell count tended to fall slowly for some weeks after the end of the severe illness.

Group III (Patients Dying after the Sixth Week). In some members of group III in whom the bone marrow failed to recover or exhibited a maturation defect, the symptoms previously described continued and the patients died after a chronic illness. Patients with severe symptoms who survived beyond the sixth week, but who ultimately succumbed, usually were emaciated.

More commonly, with late fatality, the marrow tended to recover toward the end of the sixth week, and concomitantly most of the striking manifestations of the aplastic anemia, such as purpura, disappeared, but the patient nevertheless died of pneumonitis or enteritis—either a new illness of sudden onset, or an exacerbation of a smoldering lesion that had its inception during the earlier leukopenic phase. In these patients there was now a leukocytosis and the lesions contained an abundance of polymorphonuclear cells. There was usually no longer a marked thrombocytopenia, but anemia often was profound.

Group IV (Mild Cases). Persons who were situated near the limit of the range of the radiation, or who, although close to the center, were shielded by heavy buildings, manifested mild effects. In some, who were otherwise completely asymptomatic, leukopenia was discovered during a routine blood count. Some merely complained of anorexia and malaise. Diarrhea was a common complaint. Others had mild or even severe epilation without other symptoms. When, however, purpura or oropharyngeal lesions appeared, the patients usually also had epilation and passed through a febrile illness of varying degrees of severity such as was described in its most serious form in group II.

Most patients with the milder symptoms recovered completely. There were some, however, who had not regained their feeling of well being even 3 months after the bombing. A few of these people had a persistent leukopenia of approximately 3,000 at the time that the Joint Commission left Hiroshima (January, 1946). A moderate anemia was more common. What rôle dietary factors played in the anemia is not clear nor is there any information concerning how long the depression of the bone marrow could persist.

The important symptoms of the severest cases in the various groups are epitomized in Table II, and the anatomic findings are summarized in Table III.

TABLE II

Clinical Manifestations of Severe "Radiation Effect"

Manifestation	Group		
	1st and 2nd weeks	3rd to 6th weeks	After 6th week
Epilation	o	+	+
Petechiae	o	+	o
Necrotic gingivitis and oropharyngitis	o	+	o
Diarrhea	+	+*	+†
Pneumonitis	o	+*	+†
Leukopenia	+	+	o

* Lesions usually do not contain polymorphonuclear leukocytes.

† Lesions usually contain polymorphonuclear leukocytes.

Nausea and vomiting, occurring soon after the bombing, were frequent among those who later showed other evidences of radiation effect.

Fever was a common finding in all groups before death.

SYSTEMATIC SURVEY OF THE LESIONS

In the preliminary clinical survey it has been brought out that the character of the lesions was dependent on three important factors: the date of death, shielding and distance from the bomb, and the presence of severe thermal or mechanical injury.

The possible significance of the interval between radiation injury and death in relation to dosage and susceptibility has already been considered. It is among patients dying in the first 2 weeks that the direct radiation effects as seen histologically are least obscured by the infections that are consequent upon aplastic anemia. During the month following this initial period the lesions are those of aplastic anemia of any cause together with some persistent identifiable direct radiation effects. The hemorrhages observed during this interval and for some time beyond may have a complex pathogenesis as will be outlined. After the sixth week most deaths can be considered accidents of overwhelming respiratory or enteric infection, since the bone marrow usually has recovered by this time, although it remained aplastic in occasional patients.

TABLE III

Important Anatomic Changes in Severe "Radiation Effect"

Tissue	Group I — Patients dying in weeks 1 and 2	Group II* — Patients dying in weeks 3, 4, 5, 6	Group III† — Patients dying after week 6
Adipose tissue Lung	Usually no depletion Occasional hemorrhage and edema	Occasionally depletion Necrosis and hemorrhage	Usually depletion Focal necrotizing or organizing pneumonitis

Bone marrow	A. Hypoplasia	Usually
		  A. Hypoplasia
		Occasionally
		  B. Marked reticulum hyperplasia
		  C. Focal myeloid regeneration
		  D. Marked myeloid hyperplasia
			Usually
			  C. Focal myeloid regeneration
			  D. Marked myeloid hyperplasia
			Occasionally
			  A. Hypoplasia
			  B. Marked reticulum hyperplasia

Lymph nodes and spleen	Extreme decrease of small lymphocytes	As in group I, and atypical mononuclear cells	As in group II, and occasionally regeneration of lymphoid tissue
Gastro-intestinal tract	Atypical mitotic figures and epithelial cells	Necrosis, hemorrhage, and ulceration	Necrosis and ulceration
Neck organs	Atypical mitotic figures and epithelial cells	Necrosis, hemorrhage, and ulceration	Focal necrosis and ulceration
Skin	Unknown	Petechiae and necrosis, atrophy of hair follicles	Regeneration of hair follicles; usually no other changes
Gonads (especially testis)	Incipient atrophy	Severe atrophy	Extreme atrophy

* No polymorphonuclear cells in lesions.
† Polymorphonuclear cells in lesions.
No distinction is made in this table between the direct effects of ionizing radiations and the indirect effects resulting from infection, etc. These are discussed in the text.

In others, despite hyperplasia of the marrow, the peripheral blood may continue to show aplastic anemia.

In dealing with radiation, shielding and distance factors are of prime importance. The range of effects was investigated in a large-scale clinical study in which both symptomatic and laboratory evidence was evaluated.[47] With rare exceptions, severe effects were confined to a radius of 1500 yds. about the hypocenter. The exceptions may represent inaccuracies of geographic localization, or possibly persons with an unusual susceptibility to radiation. Inaccuracies are inevitable when dealing with large numbers of persons or records, especially since some of the patients were gravely ill or unconscious and the history was obtained indirectly.

Patients with severe burns or mechanical injuries were almost never examined post mortem by the Japanese during the emergency, unless there was also evidence of radiation effect. The factors of severe burns or trauma must, however, be considered in classifying the cases since they may, by virtue of concomitant shock, infection, and other mechanisms, have contributed to the pathogenesis of various lesions found at necropsy. A "severe burn" was considered to be any of the following: (1) a first degree burn involving more than 20 per cent of the area of the body, (2) a second degree burn involving more than 10 per cent of the surface, (3) a third degree burn involving more than 2 per cent of the surface.

Since the purpose of the systematic survey is to present the pathologic data objectively, a classification of the cases has been made according to three major factors, as follows:

A. By date of death
    1. Within the first 14 days
    2. Between the 15th and 42nd day
    3. After the sixth week

B. By distance
    1. Within 1500 yds.
    2. Beyond 1500 yds.

C. By the occurrence of severe burns
    1. Without severe burns
    2. With severe burns

To simplify the systematic description of the material, a 3-digit numerical classification will be employed, one digit being drawn from each of the three major classifications as outlined above and in the order in which they have been presented. Thus the first digit refers to date of

death according to the three subheadings under that classification, the second refers to distance under the two categories that have been established, and the third according to whether or not the patient had suffered severe burns. Thus, "212" indicates a patient who died between 15 and 42 days after the bombing, who was within 1500 yds. of the center, and who had severe burns.

The cases that came under study are classified in Table IV according to the scheme just outlined. It is apparent that the 16 Nagasaki cases whose distance from the bomb is recorded are all in group III. The others will be used only for general comparison with the Hiroshima material in relation to time of death.

TABLE IV

Number and Types of Cases from Which Necropsy Specimens Are Available

Number of cases (Hiroshima)	Types of cases			Number of cases (Nagasaki)
	Identifying class number	Presence of burns	Distance	
Group I (Dead before the 15th day)				
3*	111	No burns	Within 1500 yds.	
8	112	Burns		1
1	122	Burns	Beyond 1500 yds.	
Group II (Dead 15th to 42nd days)				
58	211	No burns	Within 1500 yds.	
5	212	Burns		52
2	222	Burns	Beyond 1500 yds.	
Group III (Dead after the 42nd day)				
9	311	No burns	Within 1500 yds.	6
1	312	Burns		3
4	321	No burns	Beyond 1500 yds.	1
3	322	Burns		6
	Data not given			7
94	Grand total			76

* Material from additional Hiroshima cases, presumably in sub-group 112, although the distance was not recorded, is employed for illustrating certain lesions.

Certain gaps resulting from the fragmentary nature of some of the material will be evident in the attempted reconstruction of the sequence of events in the various systems. Obviously the reconstruction, based

TABLE V

Hiroshima, Group I: Clinical Data on Patients Dying before the 15th Day

Autopsy K no.	Day of death	Age	Sex	Distance (yds.)	Burns	Lacerations and contusions	Nausea or vomiting related to bombing	Diarrhea	Other signs or symptoms	White blood cells	Albuminuria	Fever
								Sub-group 111				
2	4	24	M	800	±±	+++	+	0	Weakness, excitement, then coma			+?
5	6	39	M	1000	±±		0	0				+?
9	8	25	M	1500	±		+	0	Headaches, simple fracture of femur	7500 (7)	+	+?
								Sub-group 112				
1	3	13	M	1300	++		+	+		9500 (7)		+
3	5	15	M	1000	++							+++
4	6	32	M	1000	+++	+	0	+++	Sleeplessness			+++
6	6	13	F	1500	+++		+		"Cerebral symptoms"			+++
7	7	34	M	1000	++		0		Simple fracture of femur	5500 (7)		+?
8	8	29	M	1200	+++		0	0				+++
11	8	25	M	1200	+++		0				+	+++
12	9	33	M	700	+++		+	+				+?
								Sub-group 122				
10	9	24	F	2000	++		+	+	Excited mental state, then coma	9500 (8)	+	++

No notation = no information given in record.

o = sign or symptom was stated to have been absent.

Burns: ±, minor burns; +, ++, +++, severe burns.

White blood cells: Figure in parentheses indicates day on which stated count was made.

Fever: +?, fever present but temperature unstated; +, fever to 39° C.; ++, fever to 40° C.; +++, fever to 41° C.

as it was on surviving material and recorded information, had certain archeologic aspects. Since the deficiencies in the records of the early cases are particularly lamentable, a special effort is made to present them in all available clinical and anatomic detail.

Group I: Patients Dying within the First 14 Days

In group I there already were profound evidences of radiation effects histologically, but epilation, purpura, and many of the spectacular indirect effects of aplastic anemia that are occasioned by the associated infections had not yet appeared. For this reason the deaths were at first clinically obscure.

The specific clinical data in the 12 patients in group I who were necropsied are recorded in Table V. There was no evidence of leukopenia at the end of the first week in the 3 patients for whom counts are available and who were stated to have been within 1500 yds. of the hypocenter. In many others, however, severe leukopenia had already

TABLE VI

Hiroshima, Group I: Heart

Observations	Sub-group		
	III	II2	I22
Gross specimens available	2	8	I
Histologic specimens available	2	8	I
Epicardial hemorrhages	2	6	
Perivascular edema	I	2	I
Thrombus in venule		I	
Plasmacytic or mononuclear cell infiltration of myocardium		I	
Plasmacytic or mononuclear cell infiltration of endocardium		I	

developed in the first few days. The actual count may have been masked by severe dehydration. Differential counts are not available for these 3 patients.

The 3 patients of sub-group III, in whom both traumatic and thermal injuries were slight, are of particular interest since they seem to have succumbed purely to the effects of radiation. They were no closer to the bomb than many others who survived. Their only protection was the wood and pantile of the Japanese buildings in which they were at the time of the explosion. The mechanism of death will be considered in the general discussion that follows the systematic descriptions.

Heart (Table VI). Petechiae of the epicardium were commonly found grossly. Microscopically, there occasionally was evidence of edema about the vessels of the myocardium. In one patient, who had been severely burned, there was plasma cell infiltration just beneath the

lining cells of the endocardium, and focal necrosis of the myocardium with mononuclear infiltration of the myolemma. Such changes cannot be considered as specific radiation effects, since they have been described in patients with many kinds of infection[31] and with burns.

Lungs (Table VII). In most instances the pulmonary changes were those of focal atelectasis and emphysema, and edema of the interstitial tissues (Fig. 20). There was no certain evidence of blast effect, although hemorrhages were observed in one instance. Among the 8 patients of sub-group 112 there was one with "neutropenic pneumonia"—focal necrosis and hemorrhage without polymorphonuclear infiltration—and another with focal pneumonia of the usual type with a heavy polymorphonuclear leukocytic exudate. In the latter instance the marrow

TABLE VII
Hiroshima, Group I: Lungs

Observations	Sub-group		
	111	112	122
Histologic specimens available	3	8	1
Focal atelectasis and emphysema	1	6	1
Edema of interstitial tissue	2	6	1
Edema fluid in alveoli	1		
Thrombi or emboli in pulmonary arterioles	1	1	
Focal parenchymal hemorrhages	1		
Focal "neutropenic pneumonia"		1	
Focal necrotizing pneumonia with polymorphonuclear cells		1	1
Additional observations			
Disseminated fibrocaseous nodules			1
Fibrous pleural adhesions		3	1
Ghon's complex		1	
Apical fibrous scar		1	

was hyperplastic although the patient (K-12) was stated to have been 700 yds. from the hypocenter. The lymphoid tissue of the lungs partook of the general atrophy which will be described. Hemorrhages tended to be more numerous in patients dying after the second week.

Hemopoietic Organs in General. In the first few days in heavily irradiated patients there was a very rapid disappearance of lymphocytes and myeloid elements, followed by proliferation of atypical cells. Some of these resembled plasma cells and others, of larger size, were intermediate in structure between the plasma cells and the large elements of the reticulum. There appeared also huge cells with irregularly folded nuclei resembling Reed-Sternberg cells.

Spleen (Table VIII). The weight of the spleen usually was less than 100 gm. In general, the capsule was wrinkled and the organ soft. The cut surface was dark red and pulpy, but little material was yielded upon

scraping. Malpighian corpuscles were invisible to the naked eye, but the trabeculae were distinct.

Microscopically, the reduction in size was accounted for by the fact that the lymphocytes had almost completely disappeared. Malpighian corpuscles were only vaguely outlined by the whorled structure of the

TABLE VIII

Hiroshima, Group I: Spleen

Observations	Sub-group		
	III	II2	I22
Histologic specimens available	3	8	1
Weight less than 100 gm.	1 of 2*	4 of 6*	1
Rupture		1	
Decrease of lymphocytes	3	5	1
Absence of germinal centers	3	5	1
Atypical mononuclear cells	3	6	1
Hyaline change in vessels	3		
Heavy mature plasma cell infiltration		1	
Perifollicular hemorrhage		1	
Numerous eosinophils		1	

* The weights of the other spleens were not recorded.

collagenous and reticular tissue that appeared as a collapsed network about the central arterioles (Figs. 28 and 29). The subendothelial tissue of the latter contained a brightly acidophilic, refractile material. The endothelium itself was well preserved. In the immediate vicinity of these central vessels were found only a few small mononuclear cells, some of which had the structure of plasma cells. The nuclei of some of the latter as well as those of certain larger cells, presumably of the germinal center, were in process of lysis. The cytoplasm of such cells had shrunk into an opaque granular mass. Among these cells there were many apparently empty spaces. The process thus appeared to be one of necrobiosis and there was little evidence of phagocytosis of lymphoid elements. However, erythrophagocytosis and hemosiderosis of moderate degree were in evidence in all of these spleens (Fig. 31). Perifollicular and intra-follicular hemorrhages were noted in one instance (Fig. 30). There was no apparent change in the fibro-elastic structure of the capsule and trabeculae. At the insertions of the latter upon the former there were deep indentations of the surface.

In most patients, despite the atrophy of the lymphoid tissue, there was proliferation of atypical cells (Figs. 30 to 32, and 40). This was already in evidence, in the earliest available specimen, on the third day after the bombing and might become so massive as to increase the weight of the spleen. The atypical cells were irregular in shape and size, and possessed neutrophilic or basophilic cytoplasm and generally hyper-

chromatic nuclei with thick nuclear membranes and prominent nucleoli. Some resembled reticulum cells, and others lymphoblasts or the "splenic tumor cells" described by Rich, Lewis, and Wintrobe.[72] Some cells were so bizarre as to suggest the Reed-Sternberg cells of Hodgkin's disease (Fig. 32). Many cells were in mitosis and some of the mitotic figures were multipolar or otherwise atypical.

In one spleen of sub-group 112, there were numerous mature plasma cells and large spindle-shaped or polygonal elements containing prominent eosinophilic granules. The pathogenesis of this change is unknown. In another patient of this sub-group, there was rupture of the spleen with a slight quantity of blood in the peritoneal cavity. This patient had sustained trauma to the head, and probably the splenic lesion was also of traumatic origin.

Lymph Nodes (Table IX). The essential changes in the lymph nodes resembled those seen in the spleen. Germinal centers were not seen and

TABLE IX
Hiroshima, Group I: Lymph Nodes

Observations	Sub-group		
	III	112	122
Histologic specimens available	o	4	o
Decrease of lymphocytes		3	
Absence of germinal centers		3	
Atypical mononuclear cells		2	

there was a striking reduction of the numbers of small lymphocytes. Thus little more than the reticular skeleton of the node remained. This compaction of the reticulum cells was clearly evident as early as the fifth day, and was particularly striking in a node from a patient who died on the tenth day (Fig. 44). Numerous mast cells were found among the reticulum cells. At 10 days a few large cells with the structure of lymphoblasts and some bizarre mononuclear elements resembling the atypical cells of the spleen had appeared. An occasional large cell was found in mitosis. These proliferative changes became more prominent in time, as will be evident from study of the nodes in group II.

Bone Marrow (Table X). Disappearance of the myeloid tissue and the presence of atypical cells within the first week after the bombing is indicated by the study of aspirated vertebral marrow,[43,52] carried out by the Japanese and later reviewed by us, in patients who may be assumed to have received a considerable dose of radiation. The failure of delivery of leukocytes to the peripheral blood was confirmed by their absence in exudates in obviously infected lesions seen at necropsy.

TABLE X

Hiroshima, Group I: Bone Marrow

Observations	Sub-group		
	III	II2	I22
Long bones			
Histologic specimens available	I	3	I
Hypoplasia	I	I	
Marked myeloid hyperplasia		2	I
Flat bones			
Histologic specimens available	0	2*	0
Hypoplasia		I	
Marked focal reticulum hyperplasia		I	

* Distance is not known in these 2 patients. Since the histologic changes in the tissues are so striking an indication of radiation effect, these patients are assumed to have been within 1500 yds.

The earliest histologic sections of marrow came from patients dying on the sixth day. Unfortunately, these were derived from long bones, where active hemopoiesis is usually not in progress in the adult. Even in these marrows there already was evidence of proliferation of the reticulum and of the formation of plasma-like cells, a process which became increasingly prominent for a time after the second week. Thus in the marrow derived from the femur or humerus of a 39-year-old man who died on the sixth day, there were small amounts of cellular tissue among the fat cells, and widely dilated blood vessels (Fig. 35). Many of the cells resembled plasma cells. In the hematoxylin and eosin preparations they had wine-colored, deeply staining cytoplasm and pyknotic nuclei. There also were other elements, intermediate in size between them and the large stellate elements of the reticulum. Some of these still retained the phagocytic property since the cytoplasm was filled with erythrocytes. Occasionally a cell resembling a reticulum cell was found to have an enormous amount of cytoplasm and a much folded vesicular nucleus. These cells were not nearly so large as the megakaryocytes and the cytoplasm was more basophilic. Only rarely was a myelocyte found at this time. There was nothing that suggested well-functioning hematopoietic tissue. Evidence of the functional insufficiency of the marrow in this case was the total absence of leukocytes in a small portion of burned skin (Fig. 13).

The first specimen of marrow from a flat bone, the sternum, was from a patient of sub-group 112, dead on the tenth day (Fig. 58), whose distance from the bomb, unfortunately, is unknown. Typical myeloid tissue was not in evidence. In its place there were numerous plasma cells and some larger atypical cells which often were found in long strings, applied adventitially to the thin-walled vessels of the marrow.

Another patient (Fig. 59), who died on the twelfth day and whose distance from the bomb is again unknown, exhibited in sections of sternum a much more striking focal hyperplasia of reticulum together with numerous plasmacytoid cells. This type of response, termed "marked focal reticulum hyperplasia," is discussed in group II and its description will not be further elaborated here.

In 2 patients, K-11 and K-12, stated to have been at 1200 and 700 yds., respectively, and who had sustained severe burns, there was myeloid hyperplasia in the long bones with "shift to the left." Granting the position of these patients to have been correctly stated, this occurrence represents an individual variation in response. Had there been no burns these patients might have been among the survivors.

Gastro-intestinal Tract (Table XI). The changes observed in the gastro-intestinal tract during the first 2 weeks included the occasional appearance, by the fourth day, in the earliest available material, of atypical epithelial cells and petechiae, and after the seventh day of occasional ulcerative lesions.

TABLE XI

Hiroshima, Group I: Gastro-intestinal Tract

Observations	Sub-group		
	111	112	122
Stomach			
Gross specimens available	3	8	1
Histologic specimens available	1	7	0
Petechiae	2	3	1
Acute ulcer		1*	
Plasma cell infiltration	1	1	
Atypical epithelial cells	1		
Small intestine			
Gross specimens available	2	8	1
Histologic specimens available	2	5	1
Ulcers and hemorrhage		1*	
Atypical epithelial cells and mitotic figures	2†	1	
Petechiae			1
Large intestine			
Gross specimens available	1	8	0
Superficial ulcers	1*	1*	
Additional observations			
Ascariasis	2	3	

* Gross only.

† There was also a Nagasaki case, K-175, dying on the 11th day, with similar changes in the epithelium, together with ulcers of the intestines.

Epithelial changes were demonstrated in a section of ileum from a patient who died on the sixth day. This material was well fixed soon after

death. The epithelium of the surface had in part sloughed, but without
loss of substance of the underlying connective tissue. That which re-
mained was extremely irregular in size and shape. Many epithelial cells
were spindle-shaped and some possessed vesicular nuclei with large
clumps of chromatin and irregular masses of basophilic cytoplasm (Fig.
80). Few small lymphocytes were left in the mucosa and there were
relatively large numbers of plasma cells together with reticulum cells.
The mucosa was edematous. The changes in the intestine of K-2, who
died on the fourth day, were similar; a tripolar mitotic figure is illus-
trated in Figure 81. Such epithelial changes were observed in the small
intestine of 3 patients, and once in the stomach. They closely resemble
lesions found in animals experimentally exposed to gamma rays.[27] Either
these atypical epithelial cells are not long viable, or else patients receiv-
ing a dose of radiation large enough to produce them do not live long,
since they were not observed in patients necropsied after the second week.

Ulcerative lesions were not observed before the seventh day. In one
patient at Nagasaki who died on the eleventh day, both bizarre, occa-
sionally binucleated epithelial cells and focal necrosis of the mucosa
and submucosa were observed (Figs. 82 and 83). Numerous bacterial
masses were found in the tissue, but there were no leukocytes. Both the
direct effects of ionizing radiation and the absence of the leukocytic
defense mechanism may have contributed to the development of such
ulcerative lesions.

Atrophy of lymphoid tissue was found in all instances in which there
had been exposure to radiation. Heavy plasma cell infiltrations, how-
ever, were found in several instances. The essentially similar changes
in the pharynx and tongue are described on page 880.

Pancreas. No significant changes were found in the pancreas.

Liver (Table XII). The liver was of the usual size. Microscopically,
the cells appeared finely granular and displayed no fatty changes. The

TABLE XII
Hiroshima, Group 1: Liver

Observations	Sub-group		
	111	112	122
Histologic specimens available	3	8	1
Giant nuclei in pericentral hepatic cells	3	1	
Central congestion, slight	3	8	1
Edema of pericentral connective tissue	3		
Heavy plasma cell infiltration of portal zones		1	

nuclei of some of the cells near the central vein were large and hyper-
chromic. The central parts of the hepatic cell cords were narrower and
the sinusoids there were more widely dilated than elsewhere. The walls

of many central veins appeared to be thick and to consist of loosely arranged collagenous material which extended outwards in strands among the cell cords (Fig. 93). This change is interpreted to be the result of edema. The Kupffer cells were not strikingly enlarged nor did they contain unusually large quantities of pigment. In one instance, from sub-group 112, there was a heavy periportal infiltration of plasma cells.

Kidneys (Table XIII). Hemorrhages were observed beneath the mucous membrane of the renal pelvis in most instances; otherwise there was nothing remarkable grossly.

TABLE XIII
Hiroshima, Group I: Kidneys

Observations	Sub-group		
	111	112	122
Gross specimens available	3	8	o
Histologic specimens available	2	7	o
Hemorrhages of pelvis	2	6	
Cloudy swelling	2	7	
Small and large mononuclear cells in corticomedullary sinusoids	1	2	
Heavy plasma cell infiltration		1	

Except for cloudy swelling of the epithelial cells of the convoluted tubules in many cases, there were no significant microscopic changes. The blood vessels did not contain fibrinoid or hyaline material. In the sinusoids of the corticomedullary junction in several sections there were remarkable collections of small and large mononuclear elements (Fig. 96). Some were small and had almost spherical, deeply basophilic nuclei and scanty basophilic cytoplasm. These resembled small lymphocytes. Mingled with them were much larger elements whose cytoplasm was neutrophilic and at times vacuolated. The nucleus was small, ovoid or bean-shaped, and its chromatin was delicately reticular. These cells had the structure of macrophages. Other cells possessed deeply basophilic cytoplasm. Their nuclei exhibited a coarse network of chromatin, with prominent nucleoli. Such cells resembled "blasts." Occasionally some of the blast-like cells were attached to the walls of these sinusoids, and transitional forms were seen which were flattened and gave the impression of being endothelial cells in process of becoming detached and rounded. Occasionally a cell was found in mitosis. There were no cells that had the appearance of mature normoblasts nor was there granulation of the cytoplasm to indicate that they were myelocytes. We have noted similar cells in similar positions in various other conditions. They have been noted also in scrub typhus.[2] In one patient who had severe burns there was a very heavy plasma cell infiltration about the glomeruli. These

cells occurred also in large numbers elsewhere in the tissues of this patient, as in the spleen and heart.

Ureters and Bladder. No changes were observed in the ureters and bladder.

Testes. Grossly, in the 4 instances in which material was available, the tubules of the testes strung out easily and no changes in size or consistency were noted.

Microscopically, in 2 of the 3 specimens available, there were striking changes, even after 4 days. The germinal epithelium had largely sloughed from the basement membrane. Sertoli cells had become prominent and in some places formed a continuous sheet. The spermatogonia were largely in the lumina of the tubules. Many were still found in mitosis, but spermatids and spermatozoa already seemed diminished in numbers (Fig. 98). Many spermatogonia and their derivatives possessed pyknotic nuclei. The Leydig cells had their usual appearance. The rete testis contained not only spermatozoa but remnants of their precursors that had sloughed away in various stages of necrosis. Such changes were noted in 2 of the 3 specimens available for microscopic examination, one each from sub-groups 111 and 112. In another of sub-group 112 the tissue was poorly preserved and no definite microscopic changes were discovered.

Ovaries. In the ovaries of one patient, 13 years of age, who probably had not reached the menarche, there were numerous primordial follicles and small follicular cysts, but no developing follicles or corpora albicantia. No other specimens were available for histologic study.

Brain (Table XIV). Striking congestion of the vessels was usually described upon gross examination of the brain. In one instance, there were superficial contusions and hemorrhages in the occipital and temporal lobes, probably of traumatic origin. In another, capillary hemor-

TABLE XIV
Hiroshima, Group I: Brain

Observations	Sub-group		
	111	112	122
Gross specimens available	2	7	0
Histologic specimens available	2	5	0
Congestion	2	4	
Edema of pia		2	
Multiple contusions with hemorrhages	1		
Capillary hemorrhages		1	

rhages were found. Histologically, neither cellular exudate nor gliosis was in evidence and the ganglion cells, including the Purkinje cells, were well preserved.

Adrenals (Table XV). In the adrenal glands of even the earliest cases there often was evidence of a decrease in cortical lipoid. Grossly, this was shown by the pale yellow-gray, rather than orange-yellow, color of the cortex and by its remarkable translucency.

TABLE XV

Hiroshima, Group I: Adrenals

Observations	Sub-group		
	111	112	122
Histologic specimens available	3	6	1
Atrophy of cortex*	1	3	1
Fibrinous material in peri-adrenal fat	1		
Heavy plasma cell infiltration		1	
Hyaline change of capsular arterioles		1	

* Usually with special involvement of the zona glomerulosa.

Microscopically, as early as the fourth day, there was evident a striking atrophy of the cells in the outer cortical zone, and edematous collagenous material extended inward from the capsule (Fig. 116). This change resembled that of the pericentral tissues of the liver (compare with Fig. 93). The other cortical cells also showed relatively little vacuolation and had finely granular cytoplasm. There was an abundance of finely granular brown pigment in the reticularis. The medullary substance was preserved in its typical histologic appearance. In another case, which was not otherwise unusual, fibrinous material had appeared in the septa of areolar tissue that traversed the periadrenal fat. Hyaline changes of the arterioles of the capsule were found in another. A third adrenal gland, from a severely burned patient, showed a striking infiltration of plasma cells.

Thyroid Gland. No changes were detected grossly or microscopically in the thyroid gland.

Parathyroid Gland. The one parathyroid gland available, from a patient in sub-group 112, was found to consist almost entirely of chief cells of typical appearance.

Pituitary Body. Three sections were available from the pituitary body, one from a patient in sub-group 111 who died on the fourth day. Despite poor fixation, it was evident that acidophilic cells predominated, which is to be expected in a 24-year-old man. The other two, one from a female and the other from a male patient, likewise showed a predominance of acidophilic cells.

Thymus. Interpretation of changes in the thymus always is difficult. The structure of the organ in a 13-year-old boy who died on the third day resembled that of the lymphoid tissue. The connective tissue was

edematous. The lobules of the parenchyma consisted of stellate reticular elements supporting smaller cells with ovoid nuclei which likewise had stellate processes. Small round cells indistinguishable from lymphocytes were rare, although they should be present in large numbers at this age. Occasional mononuclear cells with eosinophilic granules in the cytoplasm were found. Hassall's corpuscles were numerous but small. They were anuclear at their centers in most instances, and some had become calcified.

In other specimens from this group, according to the gross descriptions, almost total atrophy of the organ had occurred, but no sections were available for study.

Neck Organs. Remarkable changes had occurred in the epithelium of the pharynx, tonsils, tongue, and esophagus of K-98, the single case available for detailed study.* Unfortunately, the distance from the bomb and other exposure factors are unknown. It was stated that this man had sustained trauma and burns. It may be presumed from the appearance of the lymph nodes and the sternal bone marrow (Figs. 44 and 58) that a massive dose of ionizing radiations was received.

In the *pharynx* of this patient the epithelial cells had become remarkably swollen, vacuolated, and fragmented as had their nuclei, and much of the epithelium had desquamated. The connective tissues were tremendously edematous and there was a striking lymphectasia. Scattered plasma cells and large mononuclear cells occurred in the areolar tissue. The lymphoid tissue had become remarkably atrophic here as elsewhere (Figs. 119 and 120).

In the *tongue* the epithelium consisted of enormous, bizarre epithelial cells whose cell boundaries were now difficult to distinguish (Fig. 121). Many of the nuclei had become multi-lobed and in each lobe there was a prominent nucleolus. Multiple minute nuclei, resembling the "micronuclei" observed as a colchicine effect,[14,80] were present in some large cells. Some cells had become separated from their neighbors and appeared as refractile, homogeneous, acidophilic masses. Thick layers of parakeratotic material were present on the surface. In the connective tissue underlying the epithelium there were dilated lymphatics and small, closely crowded, spindle-shaped fibroblasts.

The epithelial cells lining the crypts of the *tonsils* exhibited changes similar to those observed in the pharynx. Here also, the nuclei were vesicular, with prominent knots of chromatin. There was a remarkable alteration of the lymphoid tissue, which was represented largely by

* Sections of the tonsils from another patient exhibited only atrophy of the lymphoid tissue but no notable epithelial changes.

compacted spindle-shaped elements of the reticulum among which were suspended only a few mature lymphocytes. The substance of the tonsil had not become necrotic.

There were notable changes in the *esophagus*. In many places the squamous epithelium of the surface had been replaced by purple-staining, dull, necrotic material. Only the swollen remnants of a few squamous cells, as bizarre in their cytoplasmic and nuclear components as those in the tongue, could still be distinguished. In the immediately under-lying tissue there was a striking degree of edema, which extended deeply among the bundles of striated muscle, and there also was dilatation of vessels. There was no leukocytic infiltration.

With certain qualities of radiation these mucous membranes are more prone to show demonstrable changes in the epithelium than the skin (Coutard[18]). Unfortunately, sections of skin from this patient (K-98) were not available.

The changes in the tissues of K-98 undoubtedly represent radiation effects. They are analogous to those in the intestine as shown in Figures 80 and 82. In the present instance, however, there were no atypical cells or mitotic figures in the stomach, which represents the only portion of the gastro-intestinal tract available in histologic sections.

Skin. Unfortunately, skin unaltered by thermal burns is not avail-able from any patient dying during the first 2 weeks.

Group II: Patients Dying During the Third, Fourth, Fifth, and Sixth Weeks

In group II, changes characteristic of radiation, such as epilation, testicular atrophy, and, particularly, damage to the hemopoietic tissues were at their peak. The most striking new developments were those associated with the infections that accompanied the aplastic anemia. All surfaces to which bacteria had access became the seat of foci of necrosis accompanied by hemorrhage. Ultimately there was evidence in many cases of generalization of the infection. If there was any leuko-cytic response, it was of plasma cell or mononuclear type. Extravasations of blood also occurred apart from any obvious local foci of infection in such organs as the kidney. The factors—heparin, platelets, bacteria, and vitamins—possibly concerned in the pathogenesis of the purpura are discussed elsewhere.[47] It is of note that the blood at necropsy often was observed to be in an unclotted state.

The salient clinical data relevant to the Hiroshima patients who ulti-mately were necropsied are recorded in Table XVI, in which all groups are compared.

TABLE XVI

Recorded Clinical Data on Hiroshima Patients, Examined by Necropsy

Observations	Group I			Group II			Group III			
	Sub-group				Sub-group			Sub-group		
	111	112	122	211	212	222	311	312	321	322
Total number	3	8	1	58	5	2	9	1	4	3
Male	3	7	o‡	46	5	1	5	1	3	2
Female	o	1	1	12	o	1	4	o	1	1
Well nourished				42	4	1	4	o	o	o
Poorly nourished				16	1	1	5	1	4	3
Burns	3*	8†	1†	10*	5†	2†		1†	1*	3†
Mechanical injury	3	1		33			5			
Nausea and vomiting on day of bombing	2	4		11						
Epilation	o	o	o	50	1		6		1	1
Purpura	o	o		38	1		2			
Ulcers or abscesses of skin	o	o		3				1		
Gingivitis (all)	o	o		37			2			
Gingivitis (necrotic)	o	o		3						
Pharyngitis or tonsillitis (all)				32			1			
Pharyngitis or tonsillitis (necrotic)	o	o		5						
Diarrhea (all)	o	5	1	24	3		4		3	1
Diarrhea (hemorrhagic)				11	1		2		2	
Epistaxis				6	3		1			
Rectal hemorrhage				1						
Vaginal hemorrhage				2						
Hemoptysis				4			1			
Fever (maximum) To 39°C.		1								
To 40°C.		1	1	21	3	1				
Over 40°C.		2		6	1		2			
Fever present, temperature unstated	2	2		17						1
Lowest white blood cell count recorded 0– 500				20			1			
501–1000				6	2		1			
1001–1500				2	2					
1501–2000							1			
2001–2500				1						
2501–3000										
Over 3000	1	2	1			1	3		1	
Lowest red blood cell count recorded Less than 1.6				2			3			
1.6–2.0										
2.0–2.5				9			1			
2.6–3.0				5					1	
3.1–3.5				6						
Over 3.5				5	3	1	1			

* Slight.

† Severe.

‡ o indicates that the sign or symptom was not present. A blank space indicates that no observation was recorded in the clinical record. The incidence of any finding as stated in the table is minimal, since the basic information may be incomplete in some instances.

Heart (Table XVII). Epicardial hemorrhages were usually, and endocardial extravasations sometimes, present (Fig. 10) and occasionally there also were hemorrhages in the neighborhood of the conduction bundle (Fig. 16). Fluid blood, which did not clot upon standing, frequently was observed in the chambers of the heart and great vessels. Perivascular hemorrhages in the myocardium occasionally were present

TABLE XVII

Hiroshima, Group II: Heart

Observations	Sub-group		
	211	212	222
Gross specimens available	55	5	2
Histologic specimens available	49	5	2
Epicardial hemorrhages*	33	3	
Endocardial hemorrhages*	6		
Fluid blood in chambers*	8	2	
Perivascular edema	4		
Perivascular hemorrhages of myocardium	7		
Thrombus in venule			1
Focal necrosis of myocardium	1		1
Plasma or mononuclear cell infiltration of myocardium	1		1
Plasma or mononuclear cell infiltration of endocardium	7	1	
Additional diagnoses			
Chronic verrucous endocarditis	2		

* Gross diagnosis.

and there sometimes was edema of the perivascular tissue, as observed in the specimens from patients dying before the end of the second week. In 5 instances, there was seen immediately beneath the endothelium of the chambers an exudate of plasma cells sometimes mingled with small and large mononuclear cells. These also were present in the myocardium (Fig. 21).

In 2 instances there was evidence of previous rheumatic disease, in the form of chronic verrucous endocarditis.

Lungs (Table XVIII). The characteristic gross lesion of the lungs in group II was that of focal necrosis centered upon minute bronchioles whose lining membranes had become necrotic. Surrounding these foci of necrosis were brilliant red zones of hemorrhage which had become confluent in some instances. The intervening parenchyma was translucent and pale, and exuded a large quantity of fluid which was only slightly cloudy. In some instances hemorrhage was predominant about extremely minute foci of necrosis (Fig. 19), but in others dull yellow or gray-green, opaque, rounded masses of necrotic tissue were surrounded by relatively narrow zones of extravasated blood (Fig. 17). Tuberculous lesions also may be surrounded by massive hemorrhages at this stage, so that vigilance was necessary in gross interpretation to avoid confusion with ordinary non-tuberculous necrosis (Fig. 18).

The pleura usually was the seat of extravasation of blood and was rendered thick and translucent by edema, which produced similar changes in the septa.

Histologically, these lungs had a characteristic structure to which the term "neutropenic pneumonia" has been applied. There was a striking degree of edema of the pleura and peribronchial and perivascular

TABLE XVIII

Hiroshima, Group II: Lungs

Observations	Sub-group		
	211	212	222
Histologic specimens available	57	5	2
Focal atelectasis and emphysema	1	1	
Edema of interstitial tissue (no other lesions)	1		
Edema of alveoli	1		
Focal parenchymal hemorrhages and edema	11*		1
Focal "neutropenic pneumonia"	31	1	
Focal necrotizing pneumonia with polymorphonuclear cells	3	2	
Focal tuberculous pneumonia	2		
Fibrinous pleurisy	4	1	
Fibrinopurulent pleurisy	1		
Hemorrhage in pleural cavity	1		
Serous pleural effusion	1		
Additional diagnoses			
Fibrous pleural adhesions	13	1	
Healed tuberculous foci	6		
Organizing tuberculous pleurisy	1		
Tuberculosis of hilar lymph nodes	2	2	
Ascaris in pleura	1		

* Six of these patients had severe ulcerative enteritis and five others had necrotizing tonsillitis to account for death.

tissues. The parenchyma showed large foci of necrosis which were centered upon the bronchioles, whose lumina were filled with finely granular, pink-staining material containing large clumps of bacteria (Fig. 22). The lining membranes of these bronchioles had become completely necrotic save that portions of the basement membrane persisted. In the vicinity of these bronchioles the shadowy remnants of the walls of the alveoli could still be discerned, but in some places these had become invisible. The alveoli were filled with fibrin, ensnaring large numbers of erythrocytes. No polymorphonuclear leukocytes were found, although large mononuclear elements with phagocytized, finely granular, brown pigment were scattered throughout the tissue.

In some instances the alveoli contained much extravasated blood (Fig. 23), but in others there were relatively few erythrocytes, but relatively much amorphous acidophilic substance, and fibrin was abundant (Fig. 24).

The lungs of 5 patients who presumably had been exposed to radiation exhibited a focal necrotizing pneumonitis in which polymorphonuclear cells were abundant. In these patients the bone marrow was found not to be hypoplastic.

Two patients had a focal caseous tuberculous pneumonitis. It is interesting that the exudate in these lungs contained numerous large mononuclear cells, although polymorphonuclear leukocytes were absent.

The caseous foci were surrounded by large extravasations of blood. Tuberculous foci in other irradiated patients of this group remained quiescent (Table XVIII).

Spleen (Table XIX). Usually the malpighian corpuscles remained in a state of atrophy which sometimes was extreme and which might possess all of the features manifest in group I, as late as the 18th day after irradiation (Fig. 42). Atypical large mononuclear cells, such as have been described, frequently were found in large numbers (Fig. 43). In some spleens there were evidences of regeneration (Figs. 41, 45, 46, and 47). The regeneration was of a remarkable type. It appeared at first to be associated with condensation of syncytial spindle-shaped reticulum cells upon the almost naked central arterioles of the malpighian corpuscles. Occasional mitotic figures were found among these reticulum cells. Small lymphocytes were seen in close association with them and indeed seemed to be derived from them. The lymphocytes appeared first between the syncytium and the central arteriole, but also in a halo

TABLE XIX
Hiroshima, Group II: Spleen

Observations	Sub-group		
	211	212	222
Histologic specimens available	51	5	2
Perisplenic adhesions	4		
Decrease of lymphocytes	48	3	1
Absence of germinal centers	49		
Atypical mononuclear cells	13	2	2
Heavy plasma cell infiltration	7		
Regeneration with perifollicular condensation of reticulum	8		
Infarct		1	

about the former. Their number increased centrally and it appeared for a time as if the "germinal center" was at the periphery of the follicle. In larger follicles, presumably later in regeneration, minute germinal centers reappeared in their central positions within the follicle.

A morphologically similar "hematopoietic perifollicular envelope" recently has been described in the spleen of the rat by Krumbhaar.[48] In the human spleen only lymphocytes appear to be derived from such cells, while in the rat they may also be precursors of normoblasts and granulocytes.

Lymph Nodes (Table XX). Between the end of the second and sixth weeks, there was usually a continued absence not only of the germinal centers of lymph nodes, but also of typical small lymphocytes. The tendency toward shrinkage on this account was counteracted by pro-

TABLE XX

Hiroshima, Group II: Lymph Nodes

Observations	Sub-group		
	211	212	222
Microscopic specimens available	39	2	1
Marked enlargement of nodes	2*	2	1
Large nodes composed of typical lymphocytes, but without germinal centers	1		
Decrease of lymphocytes	36		
Absence of germinal centers	36		
Germinal centers present	2		
Atypical mononuclear cells	25	1	1
Bacterial masses in node, with necrosis	2		
Additional observations			
Tuberculosis	2		

* K-22 and K-23.

liferation of atypical cells. In 5 instances this proliferative process was so extensive as to produce nodes of several times the usual size. Thus the bare reticular skeleton found in some of the earlier cases only rarely persisted as long as 2 weeks (Fig. 33). On the contrary, the node supported great numbers of atypical large cells, some resembling lymphoblasts (Figs. 49 and 50), some with plasmacytoid characteristics, and others representing very bizarre polymorphous derivatives of the reticulum (Figs. 51 and 52), occasionally with the structure of Reed-Sternberg cells (Fig. 53). Forms intermediate between these and ordinary reticulum cells were common. The inception of this proliferative activity was noted during the first 2 weeks, but now had reached its acme. In Giemsa-stained preparations numerous eosinophils and many mast cells of variable size and shape were present (Fig. 33). Some of the latter were remarkably elongated.

Bone Marrow (Table XXI). The tissue available is unique, since relatively little has been published concerning the effects upon the bone marrow in man of single massive doses of ionizing radiations to the whole body. Despite the devastation of the hemopoietic tissues that may occur soon after irradiation, the marrow in later weeks displayed a remarkable regenerative capacity. At first, as in the spleen and lymph nodes, the proliferative activity resulted in the production of atypical cells only. Most marrows in group II patients were of this type. In some marrows there was evidence of direct transformation of reticulum cells into myelocytes (basophilic blast cells being minimal in number), as well as into plasma cells or lymphocytoid elements as described by Rohr.[75] Some marrows, however, especially in patients dying in the fifth and sixth weeks, showed moderate or even extreme myeloid hyperplasia,

TABLE XXI

Hiroshima, Group II: Bone Marrow

Observations	Sub-group		
	211	212	222
Long bones			
Histologic specimens available	27	2	0
Type A: hypoplasia	18	1	
Type B: marked focal reticulum hyperplasia	3		
Type C: focal myeloid regeneration	5		
Type D: marked myeloid hyperplasia	1	1	
Flat bones			
Histologic specimens available	22	3	1
Type A: hypoplasia	6	2	1
Type B: marked focal reticulum hyperplasia	5		
Type C: focal myeloid regeneration	10		
Type D: marked myeloid hyperplasia	1	1	
No marrow			
Tissue response "aplastic"	5		
Polymorphonuclear cells in tissue	2		1

but even in such instances there might be evidence of a "maturation defect," with persistence of peripheral counts below 2000. The marrow of the long bones took part in the regenerative process.

The marrows of group II may be classified according to the degree and type of regeneration as follows:

Type A. Marrows showing marked hypoplasia

Type B. Marrows showing marked focal reticulum hyperplasia

Type C. Marrows showing focal myeloid regeneration

Type D. Marrows showing myeloid hyperplasia

The available material consisted of sections of tissue from either a long or flat bone, in many instances supplemented by smears made from the bones post mortem and stained by the Giemsa or Wright-Giemsa methods. If a flat bone was available, the case was classified according to the histologic features of that bone. If only material from a long bone was at hand, the case was classified according to its histologic appearance but additional information, derived from examination of supplementary smears from the rib, sternum, or vertebra, was applied when it was available.

In 8 of the patients of group II, neither marrow tissue nor smears were available. In those cases, foci of necrosis in the lung, intestine, or skin were examined to determine whether polymorphonuclear leukocytes were present. It will be recalled that even hyperplastic marrow may deliver remarkably few polymorphonuclear cells to the tissue.

The numbers in the various categories are shown in Table XXI. They will now be discussed in turn.

Type A. Some marrows still retained the almost totally aplastic character exhibited by many in the first stage, with only minimal evidence of the proliferation of reticulum and of the formation of varying numbers of plasmacytoid and lymphoid cells. This change was observed even in the ribs, sternum, and vertebrae (Figs. 55 and 56). Grossly, such a hypoplastic marrow had a gelatinous, extremely translucent, pale red appearance, and the bone yielded only a slightly cloudy, almost serous fluid upon scraping or squeezing, in contrast to the abundant, pasty, gray-red material that usually is obtained.

In some instances, with further proliferation, the reticulum and its derivatives began to form thin septa of proliferating tissues among the fat cells. Some of the reticulum cells tended to become round and to be divorced from their fellows while the nuclear membrane became thicker and knots of chromatin became more prominent in some of the larger cells. The chromatin tended to clump at the periphery and all transitional stages could be demonstrated between such elements and the typical ovoid plasma cells on the one hand and the reticulum cells on the other (Fig. 61). Rarely, and to a slight extent, there was differentiation also into myeloid elements. In most of the aplastic cases the megakaryocytes had almost completely disappeared or they might be represented by small stellate elements with vesicular nuclei and faintly acidophilic or neutrophilic cytoplasm about which fibrin tended to be deposited (Fig. 73). Islets of erythropoiesis often persisted despite extreme atrophy of the remainder of the tissue (Fig. 60). In some instances, even they had all but disappeared (Fig. 56). Clinically, these patients all had a profound leukopenia and a moderate or severe anemia.

Type B. In another type, which differs only quantitatively from that just described, there was a striking focal hyperplasia of the reticulum, and the derivative plasma cells and lymphocytoid elements might be prominent in the cellular population. There also was evidence in some instances of differentiation into myeloid cells. In part the marrow was hypoplastic, but elsewhere, particularly near the trabeculae of bone, there had occurred a tremendous proliferation of large stellate cells, whose nuclei varied somewhat in structure (Figs. 62 to 64). Many were large and ovoid, with thin nuclear membranes and delicately reticular chromatin. Others had somewhat thicker nuclear membranes and more prominent knots of chromatin. Despite the stellate shape of the associated cytoplasm, these resembled the nuclei of plasma cells, and there also were large rounded cells whose general structure was plasmacytoid and

some that resembled lymphocytes more than plasma cells. A few cells with scanty basophilic cytoplasm, thick nuclear membranes, and prominent nucleoli, which were thought to be blast cells, also were present. In Giemsa-stained smear preparations some cells were found to contain the fine azurophilic granules of undifferentiated myelocytes. Metamyelocytes were extremely rare. Evidence for the origin of the myelocytes from the stellate cells of the reticulum was the presence of cells transitional between the two, which contained a few of the azurophilic granules (Figs. 36 and 65). There were a few mast cells with numerous prominent basophilic granules closely resembling those of the lymph nodes. A few small islands of erythropoietic tissue also were found. There were also some large and small cells with complexly folded nuclei (Fig. 64). Their cytoplasm was still faintly basophilic and their structure suggested that of megakaryocytes, but some of the smaller cells were intermediate in appearance between megakaryocytes and reticulum cells.

Bacteria were found in 2 cases: streptococci in one (Fig. 66) and thin elongated bacilli in the other (Figs. 67 and 68). Both marrows were well fixed immediately post mortem. In the latter there was necrosis of the tissue, but there was no special leukocytic infiltration about the organisms in either instance. These cases demonstrate the occurrence of bacteremia, which probably was common terminally.

The tremendous focal hyperplasia of reticulum cells may suggest the appearance of "reticulo-endotheliosis" or leukemia. In that instance, involvement of the lymph nodes or other tissues rich in reticulum cells would be expected. In all cases in which "type B" marrow was encountered, however, the Kupffer cells of the liver showed little more than a moderate degree of erythrophagia and hemosiderosis, and there was no unusual proliferation of these cells. In one case with type B marrow, K-22, some of the nodes were markedly enlarged and contained numerous atypical mononuclear and reticular cells. These, however, did not differ from the atypical cells of the spleens and nodes of many other patients in whom the bone marrow was notably hypoplastic. Unfortunately, no leukocyte counts are available for K-22, but there appeared to be some differentiation of the reticulum into myeloid tissue, despite the rarity of the blast cells. It seems preferable to regard this proliferated reticulum merely as hyperplastic tissue, little differentiated, but without obvious neoplastic properties.

A single case of leukemia of monocytic type occurred among the approximately 14,000 patients studied clinically in the two cities. This case is presented in group III.

Type C. In a third type, myeloid tissue definitely predominated in

small foci where regeneration was in progress. In these foci there might still be evidence of proliferated reticulum, and excessive numbers of plasma cells and lymphocytes still were present. Myeloid hyperplasia in this instance was focal, and there was maturation at least into meta-myelocytes. Megakaryocytes appeared in increasing numbers. There was no sharp separation between the marrows of this type and those of type A or B.

Occasionally, in the Nagasaki series, eosinophils, mature and immature, were prominent in such marrows, particularly in group III (patients dying after the sixth week).

Type D. In the fourth type, seen rarely in this group, hyperplasia was extreme and the fat cells were hardly visible amid the masses of myelocytes, and in some instances metamyelocytes. The cytologic characteristics of this marrow did not differ essentially from that of type C, except for predominance of the more mature cells.

The last two types became predominant after the sixth week, and will be described and illustrated in group III.

Comparison of Tissue from Various Bones. Cell counts of bone marrow smears made at autopsy are summarized in Table XXII and in Text-Figure 1. They are grouped according to classification of histologic specimens. Thus, by reading the chart horizontally, the cytologic features of the various bones in a particular type of case can readily be compared. The columns read vertically show variations in the counts for any particular bone from case to case.

A common feature was the great increase in the reticular and lymphoid tissue, largely at the expense of the myeloid tissue. The erythroid tissue also had suffered decrease but the reduction was less in comparison with normal values.

The femur took part in the regenerative process along with the flat bones, and in some instances hyperplasia, reticular or otherwise, in the femur was extreme. Usually the smears from the flat bones conformed to the histologic structure of the long bones; in some instances there was relatively more myeloid tissue in the former. Even from long bones, however, smears showed the same contrast with fixed tissue, probably because the free, more differentiated cells are yielded more readily to the glass than are the reticular elements.

Of all flat bones, the sternum showed the earliest regeneration and the greatest cellularity, but occasionally even the femur exceeded the sternum in these respects.

TABLE XXII

Hiroshima, Group II: Comparison of Fixed Tissues and
Post-Mortem Smears of Bone Marrow*

| Cell type | Source and differential count† of smear | | | | Histology of fixed tissue |
	Sternum	Rib	Vertebra	Femur	
M	12.0	6.6			
L	31.5	43.5			Femur, type A
R	53.1	45.8			marrow
E	3.4	4.1			
	(Average of K-26, 27, 31)	(Average of K-21, 24, 25)			
M	6.0			18.0	
L	29.0			30.0	Femur, type A
R	60.0			48.4	marrow (K-30)
E	5.0			3.6	
M	21.6		7.0	7.0	
L	33.6		49.0	49.0	Sternum, type A
R	28.8		34.0	34.0	marrow (K-42)
E	16.0		10.0	10.0	
M		21.1		32.2	
L		44.0		38.2	Vertebra, type A
R		32.8		28.6	marrow (K-37)
E		2.1		1.0	
M		24.8			
L		30.5			Femur, type B
R		31.7			marrow
E		13.0			
		(Average of K-29, 44)			
M			51.5		
L			13.5		Vertebra, type B
R			27.0		marrow (K-22)
E			8.0		
M	5.0			19.0	
L	35.0			35.0	Sternum, type B
R	55.0			42.0	marrow (K-43)
E	4.0			4.0	
M	29.3				
L	21.6				Femur, type C
R	43.1				marrow (K-32)
E	5.0				

* This series of specimens is derived from patients dying during the third and fourth weeks.
† Counted by Dr. G. V. LeRoy.
  Key: M = myeloid cells; L = lymphoid cells; R = reticular cells (reticulum and plasma cells, and intermediate forms); E = erythroid cells. Types A, B, and C marrows are defined in text.

In drawing conclusions from this material, both from histologic and smear preparations, it must be remembered that one is dealing with very small and possibly unrepresentative portions of the entire vast body of the marrow.

HIROSHIMA

COMPARISON OF FIXED TISSUES AND SMEARS OF BONE MARROW
GROUP II
(Patients dying during 3rd. & 4th. week)

NORMAL

SOURCE, & DIFFERENTIAL COUNT* OF SMEAR				HISTOLOGY of FIXED TISSUE
Sternum	Rib	Vertebra	Femur	
AVERAGE OF KEY Nos. 26, 27, 31	AVERAGE OF KEY Nos. 21, 24, 25			Femur Type A
				Femur Type A (KEY No. 30)
				Sternum Type A (KEY No. 42)
				Vertebra Type A (KEY No. 37)
	AVERAGE OF KEY Nos. 29 and 44			Femur Type B
				Vertebra Type B (KEY No. 22)
				Sternum Type B (KEY No. 43)
				Femur Type C (KEY No 32)

* COUNTED BY DR. G. V. LE ROY

Text-Figure 1

Relation of Leukocyte Counts to the Histologic Structure of the Marrow. Reference to Table XVI will show that in all but one of the 34 patients in group II for whom leukocyte counts have been recorded, the count had been 2500 or less at some time before death and in most of these patients it was below 1000. This leukopenia had no relation to the type of marrow ultimately found at necropsy.

Maturation Defects. In certain instances, there was striking evidence of maturation defect, for, despite diffuse myeloid hyperplasia, peripheral counts sometimes remained remarkably low. The history of K-108 is detailed as an example.

The patient was a 29-year-old man, who was at a distance of 700 yds. from the hypocenter. He was outdoors a few paces from a concrete building. He was struck by fragments of a falling roof which inflicted slight injuries to the head and neck. There was nausea on August 6 (the day of the bombing), and on the same day he vomited between 20 and 30 times. Malaise began on August 6 and lasted until the 10th, accompanied by anorexia. He again experienced malaise beginning on August 21 until the time of death. Anorexia appeared 4 days after the second onset of malaise. There was epilation, and gingivitis on August 21, which persisted. The gingivae began to bleed on August 30. On the 25th purpuric manifestations began and there was evidence of tonsillitis, both symptoms lasting until death on September 1. There was high fever between August 24 and the time of death, and there was cough and sputum beginning on the 25th, with hemoptysis on August 30.

	Red blood cells	White blood cells
August 24	3.95 millions	370
August 26	5.64 millions	450
August 29	4.19 millions	200
August 30		220

The urine examined on August 29 was positive for albumin and negative for sugar. No statement was made concerning sediment.

The marrow of the patient, in sections derived apparently from the cavity of a long bone, was of type D, showing vascular adipose tissue crowded by very large numbers of young myelocytes. Mature polymorphonuclear leukocytes and even band cells were rare. There was an occasional megakaryocyte. Occasional cells were found in mitosis. A few small nests of shrunken nuclei, thought to be those of normoblasts, also were found. Other significant lesions at necropsy were petechiae of the skin, epilation of scalp, focal necrosis of pharynx, tongue, tonsils, and larynx, necrotizing gingivitis, an abscess in the region of the right mandibular joint, necrotizing and hemorrhagic neutropenic pneumonia, and minute hemorrhages of the gastro-intestinal tract, trachea, and renal pelves.

Rising Counts. Occasionally, however, patients who had had a profound leukopenia manifested a rise in count to approximately 5000, before death. This is demonstrated in K-86 (sub-group 211).

At the time of the bombing, the patient, an 18-year-old girl, was at home in bed at Kawaramachi, approximately 800 yds. from the hypocenter. She was injured by fragments of glass which entered both upper extremities, the left submaxillary region, the back, and the left knee joint. She lost consciousness for a time. The wounds progressed favorably, but on August 20 she noted epilation and suffered diarrhea. Suddenly in the night of August 30 there was high fever, accompanied by severe sore

throat. She was admitted to Iwakuni Naval Hospital on August 31, 1945. On that date the white blood cells numbered 960 per cmm.; on the next day, 620; then they fell daily to a minimum of 100 on September 5, 1945. After that the count gradually increased to 480, 1680, 3200, and 7980. Her general condition, however, worsened and she died on September 9.

At necropsy the bone marrow showed a moderate focal hyperplasia with myeloid differentiation (type C). The other findings included epilation of scalp, scattered petechiae of the viscera, atrophy of the lymphoid tissue, and a necrotizing focal pneumonia.

A rising leukocyte count is much more frequently recorded in patients dying later (group III). Obviously, moreover, many patients, in whom the marrow, after a period of depression, became capable of producing a leukocytosis, recovered and escaped inclusion in the autopsy series.

Gastro-intestinal Tract (Table XXIII). Hemorrhages and ulceration occurred very frequently in the gastro-intestinal tract in patients dying

TABLE XXIII

Hiroshima, Group II: Gastro-intestinal Tract

Observations	Sub-group		
	211	212	222
Stomach			
Gross specimens available	58	5	2
Petechiae	36	2	
Diffuse necrosis and hemorrhage	1		
Ulcers and hemorrhages	4		
Plasma cell infiltration	1		
Small intestine			
Gross specimens available	58	5	2
Petechiae	19		
Ulcers and hemorrhages	7		
Large intestine			
Gross specimens available	58	5	2
Petechiae	14		
Ulcers and hemorrhages	26		1
Additional diagnoses			
Ascariasis	16	1	
Chronic ulcer of pylorus	1		
Perforation (agonal) of cardia	1		
Scars of ileum	1		
Absence of appendix	1		

between the third and seventh weeks. The tissue response usually was neutropenic. In their pathogenesis these lesions probably represented the results of infection associated with the aplastic anemia, perhaps initiated in some cases by the direct action of the ionizing radiations upon the epithelium. Beyond the second week, however, the atypical epithelial cells found earlier were no longer in evidence.

Petechiae almost always were present in the stomach. They were most numerous in the vicinity of the magenstrasse, but occurred also elsewhere in the mucous membrane. Ulcerative lesions occurred occa-

sionally in the stomach, and the tissues at the base and margins of the ulcers were suffused with blood. At times necrosis and hemorrhage were diffuse, with great swelling and induration of the mucous membrane (Fig. 84). The surface assumed a dull gray-green, lusterless appearance which bordered sharply upon typical pale-pink mucous membrane. The necrotic material was underlain by a strikingly edematous and hemorrhagic mucosa. Histologically, almost no polymorphonuclear cells were found (Fig. 89) but there were numerous plasma cells in the lamina propria. Among these there were also spindle-shaped elements with nuclei resembling those of the plasma cells but with elongated bodies of cytoplasm like those of fibroblasts. Near the mouths of the glands many of the epithelial cells were found in mitosis. Most of these were in metaphase and did not appear to be atypical. At the very surface in some places there was necrosis and hemorrhage which extended halfway to the muscularis mucosae. Purple-staining bacterial masses existed in the superficial portions of the necrotic material, but no leukocytic barrier delimited the latter. The submucosa was greatly thickened by edema, and there were present large stellate elements, fibroblasts, macrophages, and mast cells. The bundles of the muscularis also were widely separated by edematous connective tissue. Irregular hemorrhages were present within the submucosa also and there were scattered lymphocytes, plasma cells, and rare eosinophils in the superficial portions of this tissue near the muscularis mucosae.

In the small intestine also there were foci of necrosis, usually discrete. They were most numerous in the region of the ileocecal valve where there was almost always involvement (Figs. 85 to 87).

In the large intestine, which was most frequently involved, the necrosis might be diffuse (Fig. 88) but the lesions usually were focal. Often there was a polypoid projection of edematous hemorrhagic tissue into the lumen of the bowel rather than an ulcer crater. The projecting mass was covered by an opaque, ashen, yellow or gray-green material, and at its base there was a halo of hemorrhage. Histologically, the tissue response again was usually aplastic (Fig. 91) or largely of the plasma cell type (Fig. 90).

Only in 4 instances were large numbers of polymorphonuclear leukocytes found in the exudate at the base of the intestinal ulcers. Most of these patients died toward the end of the fifth and during the sixth week, when the marrow was experiencing a measure of return of myelopoiesis.

Liver (Table XXIV). Contrary to the gross descriptions of the Japanese pathologists, fatty change was rarely encountered in the liver

TABLE XXIV

Hiroshima, Group II: Liver and Gallbladder

Observations	Sub-group		
	211	212	222
Histologic specimens available	55	4	1
Perihepatic fibrous adhesions	3		
Subcapsular hemorrhage	1		
Giant nuclei in pericentral hepatic cells	4		
Central congestion	24	2	
Edema of pericentral connective tissue	8	1	
Edema of periportal connective tissue	2		
Focal fatty changes: Periportal	1	2	
Midzonal	1		
Irregular	1		
Focal necrosis: Periportal	1		
Pericentral	1		
Pylephlebitis	1		
"Hyaline bodies" in cytoplasm	1		
Gallbladder			
Petechiae	1		
Additional diagnosis			
Hepatoma	1		

in this stage. It was observed microscopically in but 5 cases. Necrosis also was rare and was observed only once in pericentral and once in periportal positions. When present, the lesions were widely disseminated. The cellular exudate was scanty, had itself largely become necrotic, and consisted for the most part of plasma cells (Fig. 94). In one instance some of the large intrahepatic portal veins were involved in pylephlebitis. There was no evidence of ascariasis in that case, but there were focal necrotizing lesions of the intestine, which may well have been the source of the bacterial infection that probably was concerned in the pathogenesis of the hepatic lesions.

Slight central congestion again was frequently observed, as in group I, and there was occasionally thickening of the walls of the central veins, presumably edematous. Large nuclei were observed also in hepatic cells at the central ends of the cords.

Pancreas. No significant changes were observed in any pancreas seen grossly nor in the 25 that were inspected microscopically.

Kidney (Table XXV). Perirenal and pelvic hemorrhages were extremely common in patients dying during the second 4 weeks. Histologically, these consisted merely of erythrocytes infiltrating loose connective tissue in the capsule, and beneath the epithelium of the pelvis, without admixture of other cells. Occasionally the pelvic hemorrhages were diffuse (Fig. 97), and in some instances the ureter and bladder were involved also.

TABLE XXV

Hiroshima, Group II: Kidney

Observations	Sub-group		
	211	212	222
Specimens available	56	5	0
Hemorrhages of pelvis	33	4	
Cloudy swelling	13	1	
Glomerular hemorrhages	6		
Hemorrhages in pyramid	1	1	
Hemorrhagic pyelonephritis	1		
Scars of kidney	17		
Abscesses (with polymorphonuclear cells)		1	
Atypical large mononuclear cells in sinusoids	1		
Additional diagnoses			
Leiomyomata of pyramids	1		
Double pelvis and ureter	1		

Glomerular hemorrhages (Fig. 97) were demonstrated in approximately 10 per cent of the kidneys of patients dying during this period. Grossly, such kidneys had the "flea-bitten" appearance found in acute glomerular nephritis. The nephrons in these cases, however, merely showed hemorrhages in the subcapsular spaces and tubules, without notable proliferative changes in the glomeruli. Often a few minute scars were found, within and about which plasma cells and occasional large mononuclear cells represented the only elements of a cellular exudate. There was often cloudy swelling of the elements of the proximal convoluted tubules which, in association with anemia, accounted for the pale appearance of most of these kidneys. In one instance there was hemorrhagic pyelonephritis associated with hemorrhage and necrosis in the prostate. A few polymorphonuclear leukocytes were found in the kidney in this instance.

The large and small mononuclear cells, so prominent in the medullary sinusoids in the kidneys of patients of group I, occurred only once in the group II series.

Ureters and Bladder. In 4 instances focal hemorrhages were found beneath the mucous membrane of the ureters. Such lesions also occurred in the bladder in 3 instances, in another the process was diffuse, and in a fifth there was diffuse hemorrhage associated with an acute necrotizing cystitis.

Prostate and Seminal Vesicles. The necrotizing hemorrhagic lesion of the prostate in the patient with necrotizing pyelonephritis has already been mentioned. A few leukocytes, chiefly unsegmented polymorphonuclear cells, were present within the bladder, but they were very rare in the kidney.

The seminal vesicles showed no changes except that in one instance the contents were noted histologically to be an acidophilic material containing almost no spermatozoa.

Testes (Table XXVI). In all 38 of the available specimens in this group of 52 male patients there was atrophy of the testicular substance.

TABLE XXVI
Hiroshima, Group II: Testes

Observations	Sub-group		
	211	212	222
Well nourished patients			
Histologic specimens available	25	1	0
Atrophy of germinal epithelium and derivatives	25	1	
Thickening of basement membranes of tubules	4	1	
Hyaline changes of blood vessels	7		
Hyperplasia of interstitial tissue	2		
Malnourished patients			
Histologic specimens available	11	0	1
Atrophy of germinal epithelium and derivatives	11		1
Thickening of basement membrane of tubules	4		
Hyaline changes of blood vessels	1		
Hyperplasia of interstitial tissue	1		
Hemorrhage of tunica albuginea	1		

Since the effect of inanition* is well known, an attempt was made to determine from the records whether the patients were well nourished. Inanition usually was recorded by the Japanese pathologists or could be determined, when severe, by inspecting the subepicardial, subcutaneous, or perirenal adipose tissue in histologic sections. Twelve patients were considered to be slightly or severely malnourished; the other 26 were apparently in a good state of nutrition at the time of death. The changes to be described must also be considered in the light of the fact that some degree of testicular atrophy is common in many exhausting illnesses. In these illnesses, however, it is rarely as complete as that observed here. Most of the irradiated patients had only a short bout of high fever before death, so that this could hardly be considered a factor in the atrophy of the testes, and none had generalized lesions suggestive of typhus. Allen and Spitz[2] described testes in their scrub typhus patients that resemble those illustrated here.

Clinical sperm counts performed some 10 weeks after irradiation on survivors who were well nourished, epilated, but apparently otherwise well, showed a close correlation between the degree of exposure as judged by distance from the bomb and the degree of hypospermia. This is con-

* Testes of patients from the German prison camp at Dachau studied by Capt. E. B. Wert at the Army Institute of Pathology show all the changes described in this section. These patients weighed approximately 80 lbs. at the time of death.

firmatory evidence that the testicular changes represented at least in part an effect of radiation.

Only in one case was there a notable gross reduction in the size of the testes (Fig. 99). The masses of tubules appeared pale gray-tan but strung easily from the cut surface. Histologically, all of these testes showed separation of most or all of the germinal epithelium from the basement membrane, which was then lined by a continuous layer of tall Sertoli cells. Partly necrotic remnants of germinal epithelium and its derivatives were seen in the lumina of the tubules (Figs. 100 and 101). Spermatids and spermatozoa, however, sometimes remained embedded among the Sertoli cells. Occasionally there was found, near the basement membrane or in the lumen, a large ovoid cell with a densely reticular nucleus which from its structure was thought to be a relic of a germinal epithelial cell (Fig. 102).

Occasionally there occurred in the lumina of the tubules what appeared to be multinucleated giant cells. These apparently were formed by fusion of the cytoplasm of remnants of spermatids, successive stages of which are shown in Figures 103 and 104. Such a fused mass might become moulded into a rounded shape somewhat in the manner of a corpus amylaceum. The nuclei of such "giant cells" had a dull, homogeneous, non-reticular structure unlike that of viable cells. Barratt and Arnold[8] considered such cells in the testis of the rat after x-irradiation to be the result of atypical mitosis or amitosis of spermatocytes.

The basement membranes of the tubules within the first 5 or 6 weeks usually remained thin, but were found to be slightly thickened in a few instances (Fig. 104). In one malnourished boy of 8 years there was a remarkable thickening of the basement membrane of the tubules of the immature testes (Fig. 106). The epithelium appeared only slightly shrunken. The fate of other heavily irradiated young patients will be of great interest.

The small blood vessels of the interstitium sometimes showed deposits of a refractile acidophilic material beneath the apparently intact endothelium, with considerable restriction of the lumen (Fig. 100).

Even before there was a marked shrinkage of the tubules there might be a suggestive increase in the interstitial cells of Leydig not resulting from compaction of the tissue. This occurred uncommonly but is evident in Figure 101. It is apparently the result of hyperplasia of the Leydig cells rather than of compaction.

Changes in the hypophysis, presumably associated with the lesions in the testes, will be discussed later in this report.

Ovaries and Uterus (Table XXVII). Little suitable material from ovaries and uterus was available for inspection. There were sections of the ovaries from 10 of the 12 female patients in group II. Of these, 3 were of postmenopausal and 2 of premenstrual age. The latter usually showed numerous undeveloped follicles, some in process of atresia. In

TABLE XXVII
Hiroshima, Group II: Ovaries and Uterus

Observations	Sub-group		
	211	212	222
Ovaries			
Histologic specimens available	10	0	0
Postmenopausal	3		
Pre-menarche	2		
Ova and corpora albicantia present, developing follicles absent	4		
Marked atresia of follicles*	1		
Corpus luteum of pregnancy	1		
Uterus			
Histologic specimens available	6	0	0
Endometrium in resting phase*	3		
Postmenopausal	2		
Decidual reaction, following pregnancy	1		

* In sexually mature women.

the former there was complete involution. The four ovaries from non-pregnant women in the functional age group had in common the presence of corpora albicantia and primordial follicles without developing follicles or corpora lutea. In one young woman the primary follicles were remarkably few and those that remained were in process of atresia (Fig. 109). In some instances the ova had become shrunken in this case (Fig. 111) and granulosa cells compacted in a group at the center of the follicle. Subendothelial acidophilic deposits restricted the lumina of some vessels of the ovary as of the testis (Fig. 112).

The endometrium in all of these cases was in the "resting phase" and the central glands were lined by columnar epithelium that showed no evidence of corpus luteum effect. The spindle-shaped cells of the endometrial stroma were thin, small, and elongated.

One pregnant patient, 38 years of age (K-61) who had been in a Japanese building at 1100 yds. and who had subsequently become epilated, died on the 35th day apparently of ulcerative enteritis, shortly after she had aborted a 5 months' fetus. A corpus luteum of pregnancy was found in an ovary and there were still fragments of decidual tissue in the uterus. The resistance of the corpus luteum of pregnancy to irradiation is well known.

Neither the uterus nor its appendages escaped the hemorrhages that

involved the other tissues. They occurred in the endometrium or serosa of the uterus, the ovaries, and fallopian tubes (Fig. 108).

Brain (Table XXVIII). Congestion of the leptomeninges and intra-cerebral vessels usually was in evidence. In one instance there were large subpial, or multiple punctate subependymal hemorrhages (Fig. 113).

TABLE XXVIII
Hiroshima, Group II: Brain

Observations	Sub-group		
	211	212	222
Gross specimens available	37	o	o
Histologic specimens available	7	o	o
Subpial hemorrhages	5		
Subependymal petechiae	1		
Petechiae of cerebral substance	1		
Ecchymosis of dura	1		
Epidural hematoma	1		
Focal hemorrhage and necrosis	1		

Petechiae or ring hemorrhages were found also in several sites, including the corpus callosum, within the same brain. In another patient masses of bacteria were found within necrotic tissue surrounded by hemorrhage (Fig. 114). About these there was no leukocytic response.

There was evidence of former trauma in one epidural hematoma, but most of the hemorrhagic lesions probably had the same pathogenesis as those of the other organs.

The ganglion cells in general were well preserved, except in the vicinity of foci of hemorrhage or necrosis.

Adrenals (Table XXIX). The adrenals were almost always remark-able for their small size, which bore no apparent relation to the nutri-

TABLE XXIX
Hiroshima, Group II: Adrenals

Observations	Sub-group		
	211	212	222
Gross specimens available	58	5	2
Histologic specimens available	47	4	1
Gross evidence of loss of lipoid, no microscopic sections available	11		1
Atrophy of cortex, especially of the zona glomerulosa	30	2	
Focal necrosis	1	1	
Fibrinous material in periadrenal fat	3		
Periadrenal hemorrhages	8	1	
Hemorrhages of cortex	3	1	
Heavy plasma cell infiltration	1		
Thrombus in arteriole		1	

tional state of the patient. The outer cortex usually was remarkably narrow and was composed of translucent yellow-gray, rather than

orange-yellow, opaque tissue. This change usually was diffuse, but might be focal. The cortex at times came to resemble the gray, translucent, medullary substance. In a few instances cortical or periadrenal hemorrhages might be seen but in no instance was there a notable degree of disruption of the organ. Often, in association with the hemorrhage, there had been exudation of protein-containing material or fibrin into the areolar septa of the periadrenal fat.

Microscopically, the cells of all layers usually had finely granular rather than vacuolated cytoplasm, confirming the impression of loss of cortical lipid that was gained from the gross inspection. The cells of the deeper fascicular and reticular layers tended in some measure to retain their vacuolation. In some instances, scattered groups of cells, especially in the zona fasciculata, had extremely foamy cytoplasm and pyknotic, centrally placed nuclei. In one such, there was also focal necrosis with infiltration of large fat-filled phagocytes (Fig. 117). A remarkable finding in the same case was that of numerous mitotic figures in the epithelial cells of the central part of the zona fasciculata (Fig. 118).

Cells resembling plasma cells usually were present in small numbers in the medulla, at the corticomedullary junction, and at times also in the cortex. In one instance these cells were very numerous for no evident reason. There was no indication that they represented erythropoietic or myelopoietic tissues.

No information is available concerning the effects of castration upon the adrenals in man. In male mice, however, an increase in the thickness of the zona reticularis has been observed.[56] The pathogenesis of the adrenal atrophy observed in many of group II patients is unknown.

Thyroid Gland. Fifty-four gross and 16 histologic specimens of thyroid tissue were available. The follicles usually were small, lined by low-cuboidal epithelium, and filled with palely staining colloid.

Thymus. There were no notable changes in the 3 available specimens of thymus except for hemorrhage in one.

Pituitary Body. Twenty hypophyses were available in which the cells were well enough preserved to be recognizable as to type. Four were from female and 16 from male patients. Fixation, however, was imperfect in most of these since the capsule of the gland usually had not been opened before it had been put in the formalin solution.

Large basophilic vacuolated "castration cells" were found in 4 of the 16 male patients. In the instance illustrated in Figure 37, the basophilic cells generally were large and some had huge vesicular nuclei. In many cells vacuoles had formed in the cytoplasm and the basophilic granules were widely scattered within the trabeculae of cytoplasm. Vacuolization

was extreme in some cells which had become gigantic. The basophilic granules in them were few and the nucleus was displaced to one side in "signet ring" fashion. Cells of this type are found in most species of animals following castration.[77]

Some coarsely vacuolated basophils were found also in one female patient who had shortly before aborted a 5 months' fetus. Groups of large "pregnancy cells" were present also in this hypophysis.

In one other male and in one female patient the basophilic cells were thought to be unusually large and numerous although they were not vacuolated. However, it is difficult to draw conclusions from these sections without statistically adequate measurements and counts. Unfortunately, only single slides are available.

An interesting incidental finding in one case (K-38) was the presence of a minute chromophobe adenoma.

It is of interest to note that these changes in the pituitary body took place despite the fact that the Leydig cells remained morphologically intact and were numerous following complete atrophy of the germinal epithelium of the testes.

Neck and Mouth Organs (Table XXX). Hemorrhagic and necrotizing lesions of the mouth and neck organs were frequent at this stage,

TABLE XXX
Hiroshima, Group II: Mouth and Neck Organs

Observations	Sub-group		
	211	212	222
Gross specimens available	53	5	2
Gingivitis, all types	23		
Hemorrhagic	12		
Necrotizing	9		
Type unstated	2		
Necrotizing glossitis	3		1
Necrotizing tonsillitis	65		
Faucial	40	1	2
Lingual	25		
Necrotizing pharyngitis	13		
Necrotizing epiglottitis	21		
Hemorrhages of epiglottis	4		
Edema of epiglottis	3		
Necrotizing laryngitis	7		
Hemorrhages of larynx	4		
Edema of larynx	2		
Necrotizing tracheitis	1		
Hemorrhage of trachea		1	
Ulcerative esophagitis	2		
Petechiae of esophagus	1		
Leukoplakia of esophagus	1		

especially in the gums and tonsils. They appeared at a time when leukopenia had made the patients most susceptible to infection. What rôle

such direct radiation effects as have been described in the patients of group I play in their causation is problematic.

The gingivae became greatly swollen and infiltrated with extravasated blood. Their margins became necrotic, often with much sloughing of tissue, so that the alveolar process was exposed. Nevertheless, the teeth remained firmly attached. Pressure released quantities of hemorrhagic material from the periodontal tissues. Healing might occur with a superficial scar, resembling a zone of leukoplakia upon gross inspection. The lips as well as the lining of the mouth might become similarly involved.

Occasionally, the anterior portion of the tongue was the seat of necrosis and hemorrhage (Fig. 124), but these changes were more frequent on the dorsum where they involved the covering epithelium as well as the substance of the subjacent lymphoid follicles (Fig. 123).

More common than lingual was faucial tonsillitis. The tonsils usually were moderately and sometimes greatly enlarged, but on occasion there was no increase in size, despite total necrosis of the organ (Fig. 122). There often was complete necrosis also of tissue elsewhere in the wall of the pharynx, apart from the lymphatic tissue. Such lesions often were outlined by hemorrhagic borders and were seen also in the epiglottis, larynx, and trachea (Fig. 122).

Histologically, these lesions had in common a uniform necrosis with no barrier of polymorphonuclear leukocytes (Fig. 125) although there was a sharp line of demarcation from the relatively intact tissue. Beneath and about the lesion, however, there frequently was edema and sometimes hemorrhage. The cellular exudate, when present, consisted of scattered plasma cells and occasional small and large mononuclear elements (Fig. 126). Often, as in the tonsils, the necrosis involved lymphoid tissue, whose mature lymphocytes, as elsewhere, had almost completely disappeared and in which there was compaction of the reticulum. The reticulum had in many cases differentiated into the large atypical cells that have been described previously. In one case the lesions of thrush were observed (Fig. 127), suggesting the low state of resistance of the patient.

Skin. Of 65 patients in group II from whom specimens of skin were available, 58 were noted to have cutaneous hemorrhages at the time of death (Table XXXI). These varied from petechiae (Fig. 128) to large ecchymoses.

Ulcerative lesions of the skin not related to burns also were frequent and several were pustular. An example of an ulcerative lesion of a common type is shown in Figures 129, 131, and 132. As the region of

TABLE XXXI
Hiroshima, Group II: Skin

Observations	Sub-group		
	211	212	222
Gross specimens available	58	5	2
Epilation			
Scalp	48	2	
Axillary	8		
Pubic	6		
Eyebrows	4	1	
Beard	2		
Petechiae or purpura	53	4	1
Ulcers	20		
Pustules	3		1

denudation of the epithelium was approached, the superficial layers of cells became swollen and frequently the nuclei were found as contracted, deeply staining masses, situated within a clear cytoplasm. The deeper epithelial layers were well preserved and their nuclei were large and finely dotted with chromatin. Pigment also was present within the cells. As the margin of the ulcer was approached, the epithelium became more and more vacuolated and ultimately the nucleus was lost. In some places there was parakeratosis. The base of the ulcer itself consisted of necrotic collagen which was focally infiltrated with numerous erythrocytes. There were no leukocytes except in the septa of areolar tissue that traversed the deeper layers of the subcutaneous fat. These were of small mononuclear and plasma cell types. Even at a distance from the ulcer the skin appendages were surrounded by groups of small mononuclear cells and plasma cells. So also were the vessels of the papillae. In another instance (Fig. 130) exudate was more abundant, but it consisted largely of plasma cells and large mononuclear elements.

The mechanism of origin of the ulcerative lesions is of interest. Whether they were merely the result of infection of the skin in the patients with leukopenia or whether they were in part also the direct result of radiation is difficult to decide. The Japanese described the appearance of "blisters" of the unburned skin of some of the exposed patients. In the records of the necropsies of 2 individuals, K-98 (group I) and K-109 (group II), who were recently dead, the skin was said to have "peeled" easily revealing a pink raw surface beneath. The tongue, pharynx, and esophagus of one of these patients, K-98, showed remarkable changes in the epithelium with sloughing over large areas. This has already been described (Figs. 119 to 121). This patient died on the tenth day after exposure. Unfortunately, skin was not available and there is no supporting evidence for the concept that such ulcerative lesions are direct radiation effects in patients who died at a later date.

The only unburned skin available from most of these patients was the scalp. The changes in the surface epithelium of that region were slight, and in the patients dying later there was no evidence of telangiectatic lesions nor of any remarkable alterations in the collagen.

Epilation appeared in most instances approximately 2 weeks after the irradiation and involved men and women alike. It tended to have the same distribution as ordinary baldness in men, but in some instances the temporal and occipital regions also became epilated. Even then, a few hairs tended to remain (Fig. 9). The beard and eyebrows, and the axillary and pubic hair were relatively resistant to epilation. Regrowth of the hair usually was in progress within 8 weeks after the bombing at Hiroshima and at about 12 weeks at Nagasaki, and the new hair, initially finer, ultimately possessed the same color and texture as the old (Fig. 133).

The changes in the hair follicles are of interest and were illustrated in all phases in heavily radiated patients. If a typical hair follicle is examined (Fig. 134), it will be seen that from the matrix at the root there are differentiated strata of acidophilic trichohyalin-bearing cells (Henle's and Huxley's layers) forming an internal root sheath which is separated along a cuticle from the external root sheath. The latter is an invagination of the epithelium of the surface. Keratinization of the hair begins internally, from the trichohyalin-bearing cells of the internal root sheath very near the base of the follicle. A large keratinized shaft of hair consequently passes through the corium on its way to the surface, separated for much of its course from the external root sheath by a space.

In follicles of the scalp of the irradiated patients, however, the trichohyaline layer failed to differentiate, so that a plug of epithelium resulted which had the structure of, and was continuous with, the external root sheath. Moreover, the pigment was found irregularly distributed throughout the epithelium even at a distance from the papilla (Fig. 135). The epithelium of the matrix became flattened and less basophilic and mitotic figures among its cells became rare, whereas usually they were numerous. The matrix then formed a shallow cap rather than a long hood over the tip of the papilla. The papilla itself became less vascular and smaller and its component elongated spindle-shaped cells were compacted. Often the papilla ceased to be intimately applied to the matrix epithelium and a space appeared between the two tissues. The plug of epithelium at the base of the follicle became more and more narrow, and a whorled, pearl-like arrangement of cells might result (Fig. 136). At the same time both the glassy sheath and the external, more cellular,

fibrous membrane became considerably and often greatly thickened. Spaces appeared beneath the glassy sheath and the shrinking plug of epithelium which had become the core of the follicle. Stains for elastic tissue demonstrated that, despite its wavy refractile character, the glassy membrane was composed of collagenous rather than elastic tissue (Fig. 137). The immense thickening of the basement membranes that might occur in every follicle is demonstrated in Figure 138.

With the failure of formation of the internal root sheath, the hair shaft apparently took origin directly from the external root sheath by a process analogous to that of parakeratosis. The point of origin of the shaft came closer and closer to the surface as the matrix substance atrophied, and ultimately there remained only a bulbous plug of para-keratotic material near the mouth of the follicle (Fig. 139). This process was reflected in the structure of the cast-off hair (Fig. 140), which tapered near its formerly attached end and which was devoid of the remnants of the internal root sheath that usually adhere as a cuff.

The follicles, despite these changes, did not lose their capacity for regeneration, as shown in scalps from patients of group III (dying after the sixth week). The process of regeneration and the analogy with the normal processes of replacement of hair are described subsequently.

Other changes in the scalp were inconspicuous. There might be atrophy of the rete pegs and a general thinning of the epithelium at the surface, sometimes associated with hyperkeratosis (Fig. 138). The keratotic material was particularly abundant in the mouths of the hair follicles. Hyperpigmentation sometimes was observed in the scalps of these epilated patients. The pigment was situated in the basal layer of the epithelial cells. Atrophy of the sebaceous glands frequently was observed (Fig. 139). This process went hand in hand with atrophy of the hair follicle and cannot be interpreted as a direct radiation effect. The sweat glands in skin that had not been burned showed relatively little change, although occasionally the acini appeared shrunken and had thickened basement membranes and tiny vacuolated epithelial cells with pyknotic nuclei.

Group III: Patients Dying after the Sixth Week

The patients of group III, those dying after the sixth week, as a rule showed the effects of malnutrition. By that time the bone marrow usually had recovered or had become hyperplastic, and there was evidence of the delivery of leukocytes to the lesions in the tissue. In occasional instances, however, there was evidence of a maturation

defect, and in a few patients the marrow remained hypoplastic. Concomitantly with the recovery of the marrow, hemorrhagic lesions were relatively rare despite the necrosis of some tissues.

The important lesions at the time of death were focal necrotizing pneumonia (sometimes in process of organization) and necrotizing enterocolitis. In both of these, polymorphonuclear leukocytes usually were now prominent elements of the exudate. It is probable that the depression of hemopoiesis that occurred for a time in many of these patients was the important factor in permitting these infections to gain headway. Stigmata of radiation effect, particularly epilation and testicular atrophy, were still present, but in some patients considerable regrowth of hair had occurred that was manifest in regenerative processes histologically.

Tables XXXII to XLVI include data for group III patients from Nagasaki, since in this group information regarding distance from the bomb was stated in the protocols.

The clinical data pertinent to the group III patients who were necropsied are given in Table XVI.

Heart (Table XXXII). Aside from hemorrhages and the apparently adventitious lesions regarded as "rheumatic," and for one instance each in which gross diagnoses of "fatty change" and "focal necrosis" were substantiated microscopically, there were no significant changes in the heart.

Lungs (Table XXXIII). Multiple pulmonary hemorrhages were noted in one instance. In most cases there were necrotizing lesions of the lungs heavily infiltrated with polymorphonuclear leukocytes. In two of these (Figs. 25 to 27) abscesses had formed, with fibrous walls surrounded by large zones of organizing pneumonia. In 3 patients there again was evidence of "neutropenic necrotizing pneumonia." The marrow of one of these, who had been heavily exposed to radiation, exhibited a very remarkable degree of hyperplasia and in the other cases the marrow was aplastic. Three patients died of advanced fibrocaseous pulmonary tuberculosis.

Spleen (Table XXXIV). In the spleen, the lymphoid tissue usually was still atrophic and atypical large mononuclear cells tended to persist, but there was evidence of recovery in some cases. In these there were now abundant lymphocytes about the central vessels of the malpighian corpuscles, and in many of them germinal centers had begun to appear (Fig. 48). In certain of the corpuscles of such patients a few groups of large reticulum cells were still discernible in compact arrangement at the periphery of the collars of mature lymphocytes.

TABLE XXXII
Group III: Heart

Observations	Hiroshima				Nagasaki			
		Sub-group				Sub-group		
	311	312	321	322	311	312	321	322
Gross specimens available	9	1	4	3	6	3	1	6
Epicardial hemorrhages	4				2	1		
Endocardial hemorrhages	1							
Fatty change of myocardium	1							1
Focal necrosis of myocardium								1
Additional observations								
Fibrous pericardial adhesions	1				1			
Chronic verrucous mitral endocarditis	1			2				
Chronic verrucous tricuspid, mitral, and aortic endocarditis							1	

TABLE XXXIII
Group III: Lungs

Observations	Hiroshima				Nagasaki			
		Sub-group				Sub-group		
	311	312	321	322	311	312	321	322
Histologic specimens available	9	1	4	3	6	3	1	6
Focal atelectasis and emphysema			1	1				
Multiple hemorrhages							1	
Focal neutropenic pneumonia	2		1					
Focal necrotizing pneumonia with polymorphonuclear cells, all	6				4	2		3
Same (with organization)	2							2
Fibrocaseous pulmonary tuberculosis				2				1
Fibrinous pleurisy	1							
Additional diagnoses								
Fibrous pleural adhesions				2	1		1	1
Healed tuberculous foci	1		1					
Interstitial pulmonary fibrosis		1						
Tuberculosis of tracheobronchial lymph nodes						1		
Hydrothorax								1

TABLE XXXIV
Group III: Spleen

Observations	Hiroshima				Nagasaki			
		Sub-group				Sub-group		
	311	312	321	322	311	312	321	322
Histologic specimens available	9	1	4	2	6	3	1	5
Perisplenic adhesions	2							
Decrease of lymphocytes	6		3		5	2		2
Many lymphocytes, no germinal centers	1		1	1				1
Absence of germinal centers	7		4	1	5	1		2
Germinal centers present	2	1		1	1	2	1	2
Atypical mononuclear cells	6				3	1		1
Heavy plasma cell infiltration	1							
Focal necrosis (bacterial emboli)			1					
Infarct						1		
Hemorrhage in capsule							1	
Tuberculosis								1

Lymph Nodes (Table XXXV). The lymph nodes appeared to be even more laggard than the spleen in regard to restoration of germinal centers. They were present in the nodes of only one patient. Most lymph nodes still manifested loss of mature lymphocytes, compaction of reticulum and atypical large cells as previously described (Fig. 54).

TABLE XXXV
Group III: Lymph Nodes

Observations	Hiroshima				Nagasaki			
		Sub-group				Sub-group		
	311	312	321	322	311	312	321	322
Histologic specimens available	6	0	1	1	5	1	1	1
Decrease of lymphocytes	5		1		5		1	1
Lymph nodes composed of typical lymphocytes, but without germinal centers				1				
Absence of germinal centers	5		1	1	4		1	1
Germinal centers present	1				1			
Atypical mononuclear cells	6		1		2			
Additional diagnoses Tuberculosis of tracheobronchial or hilar nodes					1	1		

Bone Marrow (Table XXXVI). Although the marrow might remain hypoplastic, there was usually at least focal regeneration (Figs. 69 and 70), especially in the myeloid series, and sometimes marked myeloid hyperplasia (Figs. 57 and 72). This was manifest in the long bones as well as in the ribs, sternum, and vertebrae. Most patients dying at this stage had a severe anemia that might in part be associated with the omnipresent severe infections.

In some of the marrows from Nagasaki patients, relatively large numbers of eosinophilic myelocytes existed.

A typical example of a group III marrow was found in K-50. In a section of vertebrae, the marrow appeared to be hyperplastic. The cells were supported within congested and even hemorrhagic tissue. The most numerous elements in the cellular population appeared to be the younger neutrophilic and eosinophilic myelocytes. There were relatively few late myelocytes, band cells, and mature polymorphonuclear leukocytes. Occasional blast forms were seen and some cells were in mitosis. There also were many megakaryocytes and numerous small islands of erythropoietic tissue. Fairly large numbers of plasma cells, some multinucleated, also were in evidence, together with lymphocytes and large stellate phagocytes filled with hemosiderin.

In sections of rib there was still an abundance of "gelatinous marrow," within which were islands of hemopoietic tissue, again containing a predominance of younger forms (Fig. 70). The tissue was essentially

similar to the vertebral marrow except for the relatively large amounts of acellular material.

The upper half of the shaft of a femur of this patient had a remarkable gross appearance (Fig. 34). The abundant marrow contained among the stout trabeculae of bone had a translucent currant-jelly appearance in

TABLE XXXVI
Group III: Bone Marrow

Observations	Hiroshima				Nagasaki*			
	311	Sub-group 312	321	322	311	Sub-group 312	321	322
Long bones								
Histologic specimens available	6	1	3	3	1	1	1	1
Type A: hypoplasia	1		2			1		
Type B: marked focal reticulum hyperplasia				2				1
Type C: focal myeloid regeneration	4	1	1		1		1	
Type D: marked myeloid hyperplasia	1			1				
Flat bones								
Histologic specimens available	3	0	0	0	5	2	1	3
Type A: hypoplasia								
Type B: marked focal reticulum hyperplasia	2				1			
Type C: focal myeloid regeneration	1				4	2	1	2
Type D: marked myeloid hyperplasia								1
No bone marrow								
Polymorphonuclear cells in tissues			1					
Additional diagnosis								
Caseous tuberculosis of marrow								1

* The case of monocytic leukemia is not tabulated with the others.

part, but this tissue contained foci of much more opaque gray-red or pale red-brown substance. This was yielded in large friable fragments to the knife. In smears of this tissue there were clumps of young cells with coarsely reticular nuclei and abundant basophilic cytoplasm. These often were associated with islands of normoblasts and were identified as basophilic erythroblasts. There also were myelocytes and scattered plasma cells within an abundant "gelatinous" matrix.

Smear preparations of these various tissues stained by the Wright-Giemsa method were excellently preserved (Fig. 71). The only unusual feature was the presence of relatively large numbers of lymphocytes and of plasma cells, some of which were multinucleated. The granules of the myelocytes were well stained everywhere, even in the youngest forms. There were some cells resembling myelocytes, but with clear cytoplasm. These might be forms intermediate between the reticular elements and the myelocytes. Band cells and a few polymorphonuclear leukocytes also were found.

The background substance in "gelatinous marrow," as seen in K-50, deserves comment. It had a delicately fibrillar structure, which was apparent in the hematoxylin and eosin preparation. In Masson and phosphotungstic acid hematoxylin stains, this material gave the reaction of fibrin (Fig. 74). It was deposited most densely about cells, some of which had the structure of megakaryocytes. Smaller elements, more like reticulum cells, and some cells intermediate between these and mega-karyocytes also acted as centers upon which fibrin was deposited (Fig. 73).

Relation to Peripheral Leukocyte Count. Relatively few patients who lived beyond the sixth week exhibited a persistent leukopenia. In patients who did not develop a leukocytosis in response to an acute infection, the bone marrow at necropsy showed either evidence of maturation defect and was hyperplastic, as in the case which has just been described, or the marrow showed varying degrees of hypoplasia. In K-50, despite the hyperplasia of the marrow demonstrated in Figures 34 and 70, the peripheral white blood cell counts did not exceed 6500.

Laboratory Data—K-50

	Date of examination of blood			
	Sept. 19	Oct. 8	Oct. 15	Nov. 8
Red blood cells (*millions*)	2.2	1.5	1.8	1.7
Hemoglobin (*per cent*)	36	31	62	35
White blood cells	3200	5100	6500	4300
Polymorphonuclear cells, mature (*per cent*)			47.5	8
Band cells (*per cent*)			25	53
Metamyelocytes (*per cent*)			6	8
Myelocytes (*per cent*)			0	5
Lymphocytes (*per cent*)			13.5	14
Monocytes (*per cent*)			8	7
Eosinophils (*per cent*)			0	3
Basophils (*per cent*)			0	2

Reticulocytes (Oct. 15, 1945): 18 per cent
Platelets (Oct. 15, 1945): 45,900 per cmm.
Protein (CuSO$_4$ method): 6.6 gm. per cent

Notable features of the counts were the anemia, and the shift to the left in the granulocytic series. A large number of reticulocytes also was observed, indicating a regenerating but as yet insufficient erythropoietic tissue. This patient died on November 15, 1945, of a necrotizing and organizing pneumonia (Figs. 25 and 27).

In most patients surviving into the third period, there was leukocytosis associated with hyperplasia of the bone marrow, despite previous leuko-penia. K-118 (sub-group 311) is an example.

Clinical History—K-118

K-118 was a 56-year-old woman who was at 700 yds. She was thrown a distance by the blast and sustained slight contusions of the back and the legs. At the time

of the bombing she was out of doors cutting grass close to a wooden Japanese building. Few observations were recorded in the clinical history. There was nausea but the date of its occurrence was not stated. On August 26 diarrhea appeared which persisted to the time of death. It was of sanguineous type. Epilation was present but the date of onset was not stated, and there was also pigmentation of the skin. The patient was unconscious shortly after the blast and was carried to the village of Izumite where she regained consciousness. During the entire course she had only a slight fever. She died on October 5, 1945.

	Red blood cells	White blood cells
August 29	2.44 millions	1050
September 1	1.74 millions	570
September 7	3.30 millions	1040
September 15	5.31 millions	2900
September 25	3.92 millions	3600
September 29	3.72 millions	11,400
October 1	3.85 millions	17,700

The important lesions at necropsy were hemorrhagic pneumonia and ulcerative ileocolitis.

The bone marrow showed reticulum hyperplasia (type B), as illustrated in Figure 69, and there was considerable differentiation into myeloid tissue, but numerous plasma cells were still present. In the lymph nodes of this patient there was lymphoblastic hyperplasia but there was no evidence of leukemic infiltration of the tissues.

In some instances, myeloid hyperplasia in patients dying in this period was extreme and the adipose tissue of the marrow had almost completely disappeared (K-14, Fig. 72). The marrow in this case was of type D. This patient had a moderate leukocytosis before his death from acute suppurative renal disease on September 22, 1945.

A single instance of leukemia was encountered during the study of some 14,000 patients in both cities. Although this case is considered purely adventitious, it is presented in detail for the reason that the patient had been close to the bomb and had had a striking leukopenia before the leukemia developed.

Case Report*; M. Matsuo, Autopsy K-224

The patient was a 19-year-old schoolboy, who was at a munitions factory in Nagasaki, approximately 1000 yds. removed from the center. He lost consciousness and sustained a slight burn of the right leg at the bombing. A few days later he suffered from anorexia but gradually recovered. On August 28, epilation appeared and on September 3 there was fever accompanying the onset of petechiae and sore throat.

He was admitted to the hospital on September 8, when there were petechiae over the whole body, particularly upon the abdomen. The pharynx was congested but

* Abstracted, in part, from "Report of a Case of Monocytic Leukemia Occurring Following the 'Atomic Bomb Disease'" by Dr. Tando Misao, Dr. Yoshimichi Harada, and Dr. K. Hattori, Faculty of Medicine, Kyushu Imperial University.

there was no necrosis. At that time there was a marked decrease in the red blood cell count although the hemoglobin was recorded as "100 per cent." There was marked leukopenia and the sedimentation rate was increased. The nucleated cells of the marrow were found to be 22,600 per cmm. and there was an excess of lymphocytes and plasma cells. Vitamin B and liver extract were administered and the bones were irradiated with ultraviolet light. The patient improved, the white blood cell count reaching 5500 shortly before his discharge on September 30, 1945. His red blood cell count was then 3.29 millions with 77 per cent hemoglobin.

He returned to school after leaving the hospital and had no complaints. For 3 days after November 4 he worked hard at the rice harvest, but complained of a tired feeling. On the evening of November 6 there was a sensation of fever accompanied by headache, swelling of the gums with bleeding, pharyngitis with pain in the throat and difficulty in swallowing. The next day he had a high fever and there were petechiae of the extremities. On November 9 he had some sanguineous stools and was readmitted to the hospital on November 12. At that time his pulse rate was elevated to 120. There was edema and pallor of the face. Petechiae were seen everywhere in the skin and on the buccal mucous membrane. The gums were swollen. The soft palate, particularly on the right side, was remarkably swollen and was partly covered with a thick white membrane. There was gangrene of the right tonsil. Erosions were observed on the nasal mucous membrane and there was hemorrhage from the drum of the left ear. Hemorrhages were seen also on each retina. The cervical lymph nodes were enlarged to the size of a thumb and several axillary nodes of this size were palpated also. Both the liver and spleen were found to be slightly below the costal margin upon clinical examination.

Laboratory examinations showed a few red blood cells in the urine. The stools were tarry and diarrheal, and were strongly positive for blood. Hematologic examination showed a white blood cell count of 390,000 with a predominance of cells thought to be monocytes (Fig. 75). Eighty-one per cent of these cells phagocytized carbon in living preparations (Fig. 76). Two and one-half per cent of all cells were positive by the peroxidase reaction and 0.9 per cent of the monocytes were positive. The nucleated cells of the marrow numbered 566,400 per cmm., of which 91.2 per cent consisted of monocytes, including many young forms. A few of these cells were in mitosis and some vacuolated forms were found also. The hepatic and splenic punctures showed cells of similar type.

Course in Hospital. His temperature was sustained at 39° to 40° C. and the pulse rate was 100 to 120. He was given 1000 cc. of Ringer's solution, vitamins B and C, "cardiac tonics," and gargles. At approximately 1:30 a.m. on November 16, dyspnea appeared, the respiratory rate being 44 to 48, there was tachycardia of 140 to 160, and he died a few hours later in collapse.

The important laboratory and temperature data are summarized in Text-Figure 2.

Findings at Necropsy: Gross Notes

There were petechiae of the skin, pericardium, pleura, peritoneum, Glisson's capsule, renal pelves, pharynx, esophagus, large intestine, and submucosa of the oral cavity. Approximately 100 cc. of fluid blood were found in the peritoneal cavity. Small foci of hemorrhage were seen in the pulmonary parenchyma. There was necrosis of the right tonsil and a gray membrane involved both tonsillar regions and the soft palate. On the mitral valve a few verrucous masses were seen. Focal pneumonia was found in the right lower lobe. The spleen weighed 230 gm. and on the cut surface had an irregularly mottled, dark red appearance. The trabeculae were indistinct. The lymph nodes of the cervical, mediastinal, and axillary regions were enlarged as were those of the stomach and intestines. Hemorrhagic erosions and small ulcers were seen throughout the gastro-intestinal tract. Pyramidal yellow-gray zones were found in the kidneys which were thought to be infarcts.

Histologic Notes

Large numbers of mononuclear cells were found throughout most of the tissues. The cells occurred in closely compacted groups and consequently some were polygonal in outline, and an occasional cell was spindle shaped. Most of the cells presented spherical or slightly indented nuclei with prominent nucleoli. The cytoplasm apparently was much less abundant than in the cells seen in the peripheral blood. In many places groups of the atypical cells had become necrotic.

Such mononuclear infiltrations were situated among the muscle fibers of the heart,

MONOCYTIC LEUKEMIA*

MONTH						S	E	P	T	E	M	B	E	R										NOVEMBER				
DATE	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	12	13	14	15	16
DAYS AFTER BOMBING	31				35					40					45					50			53	93				97
PULSE / TEMP											DIARRHEA												DISCHARGED	READMITTED				DEATH
SUBCUTAN. HEM.	‡	‡‡	+	+	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	‡‡	‡‡	‡‡	‡‡	
GINGIVAL HEM.	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	‡‡	‡‡	‡‡	‡‡	
STOMATITIS	+	+	+	‡	+	+	+	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	‡‡	‡‡	‡‡	‡‡	
PLATELETS										180			190		204									70				
COAG. TIME (ENO)						5'0						6'0				6'0								11'0				
BLEEDING TIME			6'30	4'30						3'0			4'0		2'30		3'0			2'0				5'30				
HEMOGLOBIN	100						85			93			95		105				89		77			70				
ERYTHROCYTE (millions)	315						350			383			380		343				415		392			304				
LEUCOCYTE			1800	1650	1300	1700		2400	3800				3200	8000					4800		5300			300,000				
NEUTRO.		6.0			17.0									39.0										1.6				
EOSINO.		16.5			2.5									3.5										0				
LYMPHO.		70.0			67.0									44.5										0.6				
MONO.		5.0			10.5									9.5										979				
PLASMA		0.5			3.0									0.5										0				
NO. CELLS IN MARROW (thous.)					22.5																			8864				
NEUTRO.					25.0																			3.4				
EOSINO.					0.8																			1.0				
TOTAL					25.8																			4.4				
LYMPHO.					41.6																			1.6				
MONO.					2.2																			91.2				
PLASMA					5.2																			0.2				
RET. END. CELL.					0																			2.4				
ERYTHROID					23.4																			0.2				

* Chart compiled by: Prof. T. Miseo, M.D., Y. Harada, M.D., K. Hattori, M.D., Fukuoka

Text-Figure 2

some of which consequently became atrophic. The small blood vessels of the interstitium also were filled with atypical cells.

The walls of the alveoli of the lungs were thickened by large numbers of the atypical mononuclear cells. There also was focal necrosis about the bronchioles, whose walls had become necrotic. Polymorphonuclear cells were not seen.

In the liver the pericentral sinusoids were especially involved and there were only occasional mononuclear cells in the periportal connective tissue (Fig. 79). The hepatic cell cords at their central ends had become strikingly atrophic.

In the intestines there were tremendous submucosal infiltrations but these were fewer in the mucosa. The latter, however, had become necrotic in many large foci.

In striated muscle (Fig. 78) there was massive infiltration of cells among the fibers, some of which had become atrophic. Occasionally one of the mononuclear elements was found in mitosis. Groups of cells had suffered necrosis, but for the most part the tissue was well preserved.

In the pharynx there were striking subepithelial infiltrations. The epithelium in the section available, however, appeared intact.

Large interstitial and perivascular infiltrations were found throughout the kidneys. Many cells of the mononuclear type also occupied a subcapsular position.

The lymph nodes consisted of solid masses of the mononuclear cells such as have been described previously. There were large foci of necrosis.

The small fragment of marrow (Fig. 77) that was available contained only a few large fat cells, but there were large septa composed of masses of mononuclear cells. These had the same structure as they had elsewhere in the tissues. No islands of erythropoietic or myelopoietic tissue of the usual type were seen.

The vessels of the brain contained large mononuclear cells in massive collections; there was no interstitial infiltration in this organ.

Gastro-intestinal Tract (Table XXXVII). Ulcerative lesions of the intestine, especially of the colon, occurred often in group III. They again tended to be superficial and to be covered with fibrin. Sometimes the foci

TABLE XXXVII

Group III: Gastro-intestinal Tract

Observations	Hiroshima				Nagasaki			
	311	Sub-group 312	321	322	311	Sub-group 312	321	322
Stomach								
Gross specimens available	9	1	4	3	6	3	1	6
Acute ulcer	1							1
Petechiae	4		1	1		1	1	2
Plasma cell infiltration						1		
Small intestine								
Gross specimens available	9	1	4	3	6	3	1	6
Ulcerative enteritis	1		1		2			1
Petechiae	1						1	2
Intussusception of ileum				1				
Large intestine								
Gross specimens available	9	1	4	3	6	3	1	6
Petechiae	4		1			1	1	2
Ulcerative colitis	2	1	3	2	2	2		
Additional diagnoses								
Ascariasis	2				3		1	
Amebic colitis	1				1			
Strongyloidosis					1			

of necrosis bulged into the lumen despite destruction of the epithelium. Polymorphonuclear leukocytes might appear in the lesions. In at least one case, however, they contained relatively few of these cells despite the hyperplastic marrow, and the ulcers were histologically similar to those of the aplastic stage.

TABLE XXXVIII

Group III: Liver and Gallbladder

Observations	Hiroshima				Nagasaki			
	Sub-group				Sub-group			
	311	312	321	322	311	312	321	322
Microscopic specimens available	9	1	4	3	6	3	1	6
Giant nuclei in pericentral hepatic cells	1			1				
Central congestion, all	3	1	1	1	2		1	1
Central congestion, with necrosis	1				1		1	
Edema of pericentral connective tissue	2	1						
Focal fatty change of liver:								
Periportal	1		3	1		2		2
Midzonal	3							1
Pericentral	2							1
Disseminated					1			
Focal necrosis of liver					2			
Additional diagnoses								
Calculi in gallbladder			1					
Chronic cholecystitis					1			
Ascaris in bile duct			1					
Calculus in choledochal duct			1					
Chronic cholangiitis			1					
Tuberculosis of liver								1

An intussusception of the ileum and localized peritonitis occurred in one patient. One case of amebic colitis was found in each series in this group (Fig. 92).

Liver (Table XXXVIII). Fatty changes in the liver were much more common at this stage than in the preceding groups of patients. They probably were associated with malnutrition and other factors rather than radiation. The change usually was mid-zonal or periportal and sometimes involved as much or more than one-half of the lobule. Necrosis, apparently in association with central congestion, was in evidence in 3 instances (Fig. 95). The mechanism of the central congestion was not clear as there were no evidences of acute lesions of the myocardium in these patients, despite the verrucous endocarditis that was occasionally encountered, nor were there chronic lesions such as a significant degree of mitral stenosis to account for the congestion. However, complete and detailed gross descriptions of the heart and detailed clinical information were not available. Disseminated foci of necrosis were encountered in 2 instances.

Pancreas. No significant lesions were discovered in the pancreas. The acini frequently were small, perhaps in association with the mal-

nutrition, but otherwise the cells were typical in structure and there was no evidence of fibrosis.

Kidneys (Table XXXIX). The kidneys exhibited no specific lesions. In one case there were multiple abscesses and another showed pyelonephritis. Polymorphonuclear leukocytes were present in large numbers

TABLE XXXIX
Group III: Kidney

Observations	Hiroshima				Nagasaki			
	Sub-group				Sub-group			
	311	312	321	322	311	312	321	322
Histologic specimens available	8	1	4	3	6	3	1	6
Hemorrhage of pelvis	2	1	1		1			
Cloudy swelling	3	1	1		1			2
Scars of kidney	3	1	1		1	1		1
Abscesses (with polymorphonuclear cells)	1							
Atypical small and large mononuclear cells in sinusoids	1							
Infarcts					1			
Acute pyelonephritis						1		
Additional diagnoses								
Leiomyoma of renal pyramid	1							
Calculus of pelvis					1			1
Tuberculosis								1

in these lesions. In one patient there were the large and small mononuclear cells within the corticomedullary sinusoids that have been described previously.

Ureters and Bladder. There were petechiae of the bladder in 2 cases of sub-group 311 and in one of sub-group 312.

Testes (Table XL). In this group, all members of which were malnourished, the testicular atrophy was much more complete than in the preceding group (Figs. 39 and 105). The basement membranes usually were thickened and the Sertoli cells might be shrunken. Within the old

TABLE XL
Group III: Testes

Observations	Hiroshima				Nagasaki			
	Sub-group				Sub-group			
	311	312	321	322	311	312	321	322
Histologic specimens available*	5	1	3†	2	2	2	0	2
Atrophy of germinal epithelium and derivatives	5	1	1	2	2	2		2
Thickening of basement membranes of tubules	5	1	1	1	2	2		2
Hyaline changes of blood vessels	4			1				
Hyperplasia of interstitial tissue	3			1		1		
Infantile testis			1					
Atrophy of Leydig cells, with hyperpigmentation						1		

* All are from malnourished patients.
† One patient, 83 years of age, showed active spermatogenesis.

basement membranes there appeared in a broad band a less cellular, delicately fibrillar connective tissue. Spermatogenic tissue had completely disappeared. The tubules had shrunken and occasionally were completely hyalinized. The small blood vessels often had brightly acidophilic deposits of material beneath the endothelium and muscular wall, as seen in the earlier stages. In many cases there was now, in the opinion of one of us (A. A. L.), definite hyperplasia of interstitial tissue.

Prostate. An 83-year-old man, K-66, who was reputedly at 1800 yds., had an adenocarcinoma of the prostate. The testes of this patient, despite his age, were among the very few that showed no evidence of atrophy. In K-50, an emaciated 31-year-old man dying on the 100th day, in whom the testes were extremely atrophic, the prostate also was remarkably small grossly, as were the acini and epithelial cells histologically (Fig. 107).

Ovaries (Table XLI). In 5 premenstrual females who were within 1500 yds., numerous primordial follicles were still present. Developing

TABLE XLI

Group III: Ovaries, Uterus, Fallopian Tubes, and Vagina

Observations	Hiroshima				Nagasaki			
	Sub-group				Sub-group			
	311	312	321	322	311	312	321	322
Ovaries								
Histologic specimens available	4	o	1	1	3	o	1	3
Postmenopausal	1		1				1	1
Premenarche	1			1	2			1
Developing follicles absent,* corpora albicantia present	2				1			1
Hemorrhages							1	
Uterus, fallopian tubes, and vagina								
Histologic specimens available	1	o	1†	o	1	1	o	1
Endometrium in resting phase	1				1			
Myomata			1					
Endometriosis						1		
Acute salpingitis						1		
Tuberculosis								1
Chronic vaginitis								1

* In premenopausal mature women.
† Postmenopausal.

follicles were absent in 3 in whom both corpora albicantia and primordial follicles were in evidence. Atrophy was much less evident than in the male.

Brain (Table XLII). Suppurative complications in the brain were relatively frequent. In this group there were 2 patients with suppurative meningitis and one with a "cerebral abscess." The abscess might possibly have been a tuberculous lesion since the patient had caseating pulmonary tuberculosis, but histologic sections were not available.

TABLE XLII
Group III: Brain

Observations	Hiroshima				Nagasaki			
	Sub-group				Sub-group			
	311	312	321	322	311	312	321	322
Gross specimens available	8	1	4	3	6	2	1	6
Histologic specimens available	5	0	3	1	1	0	0	0
Petechiae of cerebrum	1		1					
Suppurative meningitis with polymorphonuclear cells	2							
Abscess of occipital lobe*				1				
Thrombus in superior longitudinal sinus	1							
Additional diagnoses								
Senile plaques			1					
Microgyria			1					
Cystic change of lenticular nucleus			1					

* Gross specimen only.

Adrenals (Table XLIII). The atrophy that has been noted previously in the adrenal glands was extreme and in some cases involved all layers (Fig. 115). Usually the outer portion of the zona glomerulosa showed the most striking changes. Again it must be emphasized that most of these patients were emaciated.

TABLE XLIII
Group III: Adrenals

Observations	Hiroshima				Nagasaki			
	Sub-group				Sub-group			
	311	312	321	322	311	312	321	322
Gross specimens available	9	1	4	3	5	3	1	5
Histologic specimens available	8	1	3	3	5	3	1	5
Gross evidence of loss of lipid, no microscopic sections available			1					
Atrophy of cortex, especially of the zona glomerulosa	6	1		1	5	2	1	5
Focal fatty changes of cortical epithelium			1		1	1		
Focal necrosis of cortical epithelium			1			1		
Periadrenal hemorrhages		1					1	
Hemorrhages of cortex	1		1				1	
Chronic passive congestion	2							
Additional diagnosis								
Tuberculosis							1	1

Thyroid Gland (Table XLIV). Occcasionally in these emaciated patients the thyroid showed variation in the size of the follicles. In such thyroids there were many minute follicles lined by low-cuboidal epithelium, and thick septa of connective tissue infiltrated with lymphocytes traversed the organ. The significance of this lesion is difficult to assess. Four of 6 patients showing this change were stated to have been beyond 1500 yds.

Pituitary Body. Six pituitary glands were available in histologic sections. In K-50 the basophilic cells were remarkable for their large

size. A few were vacuolated. They were present in groups in all parts of the gland. The eosinophilic cells, on the contrary, were minute and inconspicuous. The changes in the pituitary body were much less striking than in K-42, Figure 37. The testis of K-50 is illustrated in Figure 105.

TABLE XLIV
Group III: Thyroid Gland

Observations	Hiroshima				Nagasaki			
		Sub-group				Sub-group		
	311	312	321	322	311	312	321	322
Gross specimens available	8	1	4	2	5	3	1	6
Histologic specimens available	4	0	0	1	4	2	1	5
Slight increase of interstitial tissue, and lymphocytic infiltration	1			2	1			2

TABLE XLV
Group III: Mouth and Neck Organs

Observations	Hiroshima				Nagasaki			
		Sub-group				Sub-group		
	311	312	321	322	311	312	321	322
Histologic specimens available	7	1	4	2	6	3	1	6
Gingivitis, all types	3				1			1
Hemorrhagic	2							
Necrotizing	1				1			
Necrotizing focal tonsillitis	4	1	2	1				
Scars of tonsils	1							
Chronic glossitis								1
Laryngitis					1			

One other pituitary body showed slight vacuolation of the basophils, whose number, however, was small. The patient was a 78-year-old woman. This change was found constantly in senile females.[77]

Mouth and Neck Organs (Table XLV). Necrosis of the tonsils sometimes occurred in this group, but it usually was focal rather than diffuse. In one case microscopic sections showed evidence of necrosis without leukocytic infiltration, but there was hypoplasia of the bone marrow. In others, in which the marrow was not aplastic, polymorphonuclear cells were found within the necrotic tissue lining the crypts. There was no hemorrhage. Necrotizing gingivitis occasionally was still in evidence. There usually was no necrosis of the larynx or epiglottis.

Skin (Table XLVI). Petechiae in the late cases were unusual. Hair follicles often showed evidence of regeneration. There were renewed differentiation of the internal root sheath, regrowth of the papilla, decrease in the thickness of the basement membrane, and a new hair shaft was found on its way outwards through the plug of keratinized epithelium at the mouth of the follicle. A completely atrophic follicle showing all of these changes described in group II is illustrated in Figure 141. In

TABLE XLVI
Group III: Skin

Observations	Hiroshima				Nagasaki			
	Sub-group				Sub-group			
	311	312	321	322	311	312	321	322
Gross specimens available	9	1	4	3	6	3	1	6
Epilation	5		1	1	2	1		3
Hemorrhages					3	2		1
Decubital ulcers		1		1	3	2		2
Eczema			1					

Figure 142 are shown the beginnings of the regenerative process. This process of atrophy and regeneration was a recapitulation of the usual processes of loss and replacement of the hair. It has been described in detail by Auburtin[6] (Fig. 143). In the adult, hair usually grows from the same root for 3 or 4 years. Then there is failure of differentiation of the internal root sheath, the basement membrane thickens, pigment becomes irregularly distributed, and the hair is extruded, just as in the irradiated persons. Regeneration takes place apparently from the same follicle and the keratinized tip of the new hair, capped with a new internal root sheath, burrows its way through the old plug at the mouth of the follicle. In the irradiated person the process was condensed in time and involved the great majority of follicles. Perhaps also in these follicles the atrophy was more complete, the thickening of the glassy membrane was greater, but regeneration nevertheless occurred. Regeneration was evident clinically in individuals with severe epilation within 7 weeks after the explosion.

In emaciated individuals of this group decubital ulcers occasionally occurred, as was to be expected.

DISCUSSION

Mechanism of Death

The factors responsible for death from radiation effect are not entirely clear, especially in those patients dying within the first 2 weeks. It is possible experimentally with x-rays to produce deaths "under the beam" if the dose is delivered at a sufficiently rapid rate.[39,69,83] Henshaw[89] has demonstrated histologically the widespread damage to tissue that may occur within 3 hours in animals receiving 25,000 to 50,000 r. at a rate of 250 r. per minute. Whether any of the population at Hiroshima and Nagasaki undamaged by burns or trauma died of such direct destruction of tissue within the first few hours is not known, since the first autopsies were not performed until 3 days after the bombing. During these first few days no clinical records of blood pressure were made, nor of fluid

balance studies. Thus the contribution of "shock" to radiation sickness cannot be evaluated.[60]

There is evidence of widespread damage to tissue of the same histologic pattern as that seen in animals succumbing within the first day after overwhelming doses of radiation. In the absence of bacteriologic studies, however, it is not possible to state with certainty that bacteria did not contribute to the early mortality, for even minor injuries and burns did not often escape infection. At 11 days the intestine of a patient at Nagasaki already showed large masses of bacteria lying within necrotic and edematous tissue in the mucosa (Fig. 83).

Few experimental studies have been concerned with the study of the generalization of infection after irradiation. Warren and Whipple[93] did not find evidence of an overwhelming bacterial invasion from the intestines of dogs dying within the first 4 days. Chrom,[16] using heavy doses of x-rays, found bacteria localized to the mesenteric lymph nodes within the first few days and generalization only after the seventh day. Similarly, after neutron or x-ray irradiation of animals, Lawrence and Tennant[49] found the blood and tissue cultures to be sterile within the first 4 days of radiation, but that "as the doses are decreased and the animals live longer, bacteremia is a usual finding and infection probably a more important factor in the cause of death."

After the second week heavily infected necrotic and ulcerated lesions in the skin, mucous membranes, and respiratory tract were invariably observed, and there was also in some instances, although cultures were not made, morphologic evidence of generalization of the infection with masses of bacteria in freshly fixed organs as remote from the surface as the brain (Fig. 114), bone marrow (Figs. 66 to 68), and eye.[95] Thus, in those patients who survived longer, infection appears to have become an increasingly important factor. Even localized infections can, under certain circumstances, contribute to the depression of bone marrow with resultant leukopenia and anemia.[19] Many of the lesions are indistinguishable from those seen in patients with aplastic anemia of other causation where, again, bacterial infection likewise is of primary importance in the mechanism of death. Infection, once established, can also affect the regenerative process in the tissues.

The definitive answer concerning the rôle of bacteria in increasing mortality and preventing recovery following irradiation will not be forthcoming until aseptically reared animals are exposed.

The interpretation of the histologic changes observed in these patients is rendered difficult not only by the factor of infection, but by the possible

influence of one damaged organ upon another. As far as hemopoiesis is concerned, Lawrence, Valentine, and Dowdy,[50] in cross-circulation experiments, found no evidence of an immediately operative humoral effect. Experiments of this type, however, do not completely solve the problem. An example is the appearance of "castration cells" in the pituitary body after destruction of the spermatogenic tissue of the testis. The careful analysis necessary before ascribing any changes to the direct action of ionizing radiations is well illustrated by the series of studies of the adrenal of the rabbit carried out by Engelstad and Torgersen.[23,25,81] In the last of a series of papers, Torgersen[81] concluded that the changes originally ascribed to the irradiation of the adrenal were in fact brought about indirectly. The histologic changes themselves that are observed after radiation are not specific, and every change can individually be produced by some other means.

Mitosis and Cell Activity

Some of the effects of ionizing radiation on mitosis and activity of cells are now well known. The first autopsies came too late to detect whatever effect there may have been upon suppression of mitosis, which is stated by some to be a sensitive indicator of the action of ionizing radiations.[87] During the first few days many of the mitotic figures in the intestine, spleen, and elsewhere were atypical, and numerous bizarre cells, some with giant nuclei, appeared in these tissues. In the hemopoietic tissues the cells were so atypical as to resemble those found in Hodgkin's disease. "Micronuclei" appeared as in cells under the influence of colchicine.[14,80] Similar effects on mitosis and the presence of atypical cells have frequently been noted in the heavily radiated tissues of animals and men.[26,57] It is probable that most of these cells are not long viable, since after the second week they became increasingly rare in the tissues of the atomic bomb patients. Maximow[57] came to the same tentative conclusions in his studies of irradiated connective tissue, although he did not consider them definitive. The proliferative capacity of many tissues, however, if at all damaged, was soon restored, as demonstrated by the reticulum cells in the hemopoietic tissues, and the Sertoli cells in the testes. Phagocytic activity likewise was in evidence in the ingestion of red blood cells by the reticulo-endothelial cells of the bone marrow and spleen. Subsequently hemosiderosis developed, as described in the early observations of Heineke.[84] Again the factor of infection must be taken into account in considering the pathogenesis of this process.

The Blood-Forming Organs

The sequence of changes in the peripheral blood in the population exposed to the atomic bomb was, in general, similar to that known to occur in animals and men exposed to adequate rapidly administered doses of ionizing radiations, as contrasted with repeated minute doses.[44,53] The first counts were made too late, however, to observe the initial leukocytosis so commonly found in the first few hours[21,37,38,59] in irradiated animals and patients. The initial polymorphonuclear (and with relatively small doses, lymphocytic[37]) leukocytosis probably represents a "mobilization rather than new formation"[11] of cells.

The first counts in the patients, made at Nagasaki on the first day after the bombing and at Hiroshima on the fourth, already indicated a leukopenia which became increasingly severe during the succeeding weeks.[47] The platelet counts fell later than the leukocyte counts and the erythrocyte levels sank more gradually than either.[21,69] Despite the very severe aplastic anemia, young forms of the white and red blood cells sometimes appeared in the peripheral blood during the first 10 days.

The disappearance of mature lymphoid cells from the lymph nodes, spleen, and thymus was striking even in the first available sections of the fourth day. Experimentally, during the administration of massive doses of x-rays, Henshaw[89] found striking destruction within the first 3 hours after the radiation was started. In the atomic bomb patients the cells seemed to undergo autolysis *in situ* and there was little evidence of their phagocytosis as described by Tsuzuki,[83] Henshaw,[87] and others. The slow restoration of the lymphocyte count was associated in these patients as well as in experimental animals with the slow recovery of the lymphoid tissues observed histologically.[11,21]

Similar rapidly destructive changes in the bone marrow were produced by Henshaw[89] within 3 hours during massive irradiation. In the patients, tissue from a cancellous bone was not available until the tenth day, but at that time there was almost total loss of the usual hematopoietic substance. In the subsequent 3 weeks, some marrows showed persistence of erythroblastic foci despite disappearance of granulopoietic tissue. This finding in irradiated bone marrows has been reported by Dunlap.[21] Bloom's[10] statement that the erythroblasts are exceedingly sensitive was not confirmed in the present material.

Remarkable was the resistance to destruction of the reticulum cells. A skeleton of these elements remained in the lymph nodes, skin, and bone marrow despite complete destruction of all mature cells. This

radio-resistance of the "macrophage system" has been noted by Bloom.[10]
The reticulum cells retained their morphologic integrity even after the
devastating doses employed by Henshaw.[39] Remarkable also was the
regenerative capacity of reticulum cells. Proliferation of these elements
was already in evidence by the sixth day in the earliest available speci-
men of a long bone. Different patients varied in the extent of regenera-
tion, but when it occurred, the long as well as the flat bones took part
in the regenerative process. The large agranular pale cells might form
focally or diffusely distributed masses of stellate or rounded elements.
They were described very early by Heineke[36] in regenerating marrows
following external radiation, and by Martland[55] in marrows of patients
who had ingested radium or mesothorium. Martland considered this
"regenerative leukopenic anemia" the result of internal, as contrasted
with external, radiation which in his view resulted in an aplasia of the
marrow. This is certainly belied by the appearance of the marrows of
many patients dying some 6 weeks after the atomic radiation. Hyper-
plastic bone marrows likewise may occur in individuals with "aplastic
anemia" not produced by ionizing radiation, as described by Rhoads and
Miller,[71] who also described large pale cells similar to those of the early
phase of regeneration after the atomic bomb radiation. In the heavily
irradiated patients these cells appeared at first to differentiate by a
gradual series of transformations into plasma cells. After the first
month, however, such cells appeared in some patients to acquire
azurophilic granules and to be connected by a series of transition
forms with myelocytes. Often deeply basophilic blast cells were present
in such marrows, but in relatively small numbers, suggesting that the
reticulum cells can be transformed quickly into myelocytes. Later,
hyperplastic marrows of more usual structure, but retaining for some
months unusually large numbers of plasma cells and lymphocytes,
might be seen. This sequence of events was actually traced by repeated
bone marrow aspiration studies in a series of patients[47] and is not de-
scribed merely from the histologic appearance of bone marrows of pa-
tients dying at various times after the irradiation.

As Dunlap[21] and Rhoads and Miller[71] have pointed out, the cellu-
larity of a bone marrow is not necessarily an index of the rate of delivery
of cells to the peripheral blood. The nature of the "maturation defect"
in some of the hyperplastic marrows is completely unknown. Perhaps
the "defect" lies in the supply of some essential substance, as in perni-
cious anemia, or possibly the infections that accompany the phase of
severe depression of the marrow are responsible. Certainly a major

therapeutic effort should be made to eliminate the factor of infection pending the resumption of more orderly hemopoiesis.

The mechanism of hemorrhage after irradiation recently has been illuminated by the studies of Allen and Jacobson[4] who demonstrated the appearance in the blood of a substance with the properties of heparin. This work, unfortunately, was unknown to the physicians in the field at Hiroshima and Nagasaki. It must be stated, however, that these observers were puzzled by the appearance of hemorrhages in patients whose levels of platelets had not fallen to values when hemorrhages usually occur.[47] The source of the heparin is unknown although mast cells were found early in relatively large numbers in the bone marrows and lymph nodes of the irradiated patients and later in the submucosa of the intestines beneath ulcerative lesions. The presence of mast cells in large numbers in the tissues is not necessarily indicative of hyperheparinemia, as Oliver[66] has shown in his observations of dogs with mast cell tumors. Toward the end of the first month, however, platelets in patients with a severe radiation effect often fell below 10,000 per cmm., a level at which cutaneous hemorrhages could be expected. The importance of thrombopenia as such in the mechanism of hemorrhage has likewise been recognized by Allen and his co-workers.[3]

Eosinophilia occasionally has been found in animals and patients after irradiation and was observed in some of the Nagasaki patients. The mechanism of the eosinophilia is obscure. There is some question, however, as to whether there may not have been exposure to renewed hookworm or other infections by parasites in these patients, who were thrust into unhygienic surroundings after their displacement from the city. It is interesting to note that Jacobson and Marks[44] found no evidence of eosinophilia in animals irradiated daily for long periods with 0.11 to 8.8 r. of gamma rays.

The Gonads

It was realized from the first by Albers-Schönberg,[1] the discoverer of the sterilizing effects of x-rays, but often lost sight of by his successors, that mere histologic changes in the testes must be distinguished from the condition of sterility by the test of mating after an adequate interval of time. As Warren[88] has stated, the testis is not far different histologically whether in temporary or in permanent azoospermia; in the former condition a lesser number of germinal epithelial cells appears to have been destroyed. In the patients exposed to the atomic bomb, the testes showed profound destructive changes beginning as early as the

fourth day when the first material was available for histologic examina-
tion. The changes became more profound in succeeding weeks, but ad-
ditional factors—infection and starvation—probably became concerned
in the pathogenesis. In many of these patients a few spermatogonia still
remained in close association with the basement membrane. A controlled
clinical study was performed some 10 weeks after the explosion by ex-
amining active and well nourished men who had been relatively close
to the center. In many of these, sperm counts revealed hypospermia and
azoospermia in contrast with the findings in a group of prisoners who
had been just beyond the range of radiation. Follow-up studies on the
exposed men will be necessary, however, to establish whether perma-
nent or temporary azoospermia has been produced.

The relation of the testicular atrophy to the appearance of "castration
cells" in the pituitary body is of interest. Similar changes in the pitui-
tary body have been observed in the rat after irradiation of the testes.[46,86]
In this process the interstitial tissue of the testes remains morphologi-
cally intact or even becomes hyperplastic. This suggests that the germi-
nal epithelium or its derivatives may have an endocrine function. Further
evidence is the appearance of "castration cells" in the pituitary body
after ligation of the ductuli efferentes.[85]

The relative sensitivity to irradiation of the human ovary and testis
has not as yet been finally established. No additional conclusive infor-
mation was adduced on examination of tissues of patients exposed at
Hiroshima and Nagasaki. The histologic changes were less spectacular
in the ovary. The incidence of amenorrhea in the women was found to
be inversely proportional to their distance from the center, but data of
this type are less objective than sperm counts in the men and are some-
what obscured by the high incidence of "war amenorrhea"[47] in Japan,
and perhaps by uterine bleeding of other than menstrual origin such as
may accompany purpura. According to Dunlap,[22] the sterilizing dose
for men is probably less than that for women. Under the conditions of
irradiation described by Peck and his associates,[67] it was possible to
produce permanent amenorrhea in 50 per cent of women following the
administration to the tissues of 125 to 150 r. Above 625 r., almost all
female patients were permanently castrated. It seems possible that non-
fatal doses of radiation to the body as a whole may produce permanent
sterility in some instances. On the other hand, even massive doses to
the ovary may not result in permanent amenorrhea.[45] It is interesting
to note, moreover, that conception can take place in some patients de-
spite post-irradiation amenorrhea.[58] Species differences may exist in

the relative sensitivity of the gonads of the two sexes. Thus, in the mouse, Lorenz and his associates[53] found that upon protracted irradiation with small doses the injurious effect was cumulative and irreversible for the ovary but not for the testis.

The Fetus and the Germ Plasm

It is now well known from clinical experience that irradiation may exert damaging effects on the unborn child without producing abortion. The resistance of the corpus luteum is well known.[73] After the application of a 60 per cent skin erythema dose (600 r. by his technic) to the tissue of the ovary, Mayer and his co-workers[58] found that abortion could be induced in 96 per cent of pregnant women. If an abortion does not result after large doses of radiation to the pelvis, the incidence of microcephaly and idiocy in a child is high, especially if the irradiation is administered before the end of the fourth month of pregnancy.[22] No statistics are available concerning such occurrences among the Japanese after the atomic bombings. The disrupted municipal facilities prevented accurate collection of vital statistics. It is probable that infant mortality was high because of the appalling hygienic conditions that prevailed after the atomic bombings and the great typhoons that followed.

Non-sterilizing irradiation of the female pelvis, when carried out before conception, is stated not to be followed by an unduly high incidence of abnormal offspring.[22,30,63,64] The studies concerning fertility made to date by questioning radiologists[42] or technicians[65] are inconclusive, since large masses of data are required together with adequate control material.

Effects on the germ plasm, as has been pointed out by the pioneer studies of Muller[61,62] on fruit flies, and by Snell[78] of Muller's laboratory on mice, must be studied with care over a series of generations before conclusive evidence can be forthcoming. These changes in the germ plasm are entirely distinct from those that can be produced in the embryo by radiating the products of conception. Muller[61] and others have demonstrated changes of two types in the germ plasm itself consequent upon irradiation: (1) gene mutations, by which is implied a change in the actual composition of the genes, and (2) chromosome transformation, whereby is implied a change in the position of the genes within the chromosomes. Such gene mutations as may result are usually recessive, and can be detected no sooner than in the third generation, and then only by inbreeding. Such mutations are more apt to be manifested by the elusive effects of lower "general vitality or efficiency than conspicuous

morphological abnormalities."[62] The most common situation, moreover, is for the gene or chromosome mutations to be lethal to the embryo. These fundamental facts were established for Drosophila by Muller and have been confirmed by others for many species. In mammals, Snell,[78] working with mice, found evidence of translocation changes in the chromosomes manifested by heritable lowered fertility ("semi-sterility"). Small litters developed in stocks the male ancestors of which had had their testes exposed to doses of between 600 and 800 r. Such heritable changes can be produced by irradiating mature spermatozoa as well as the spermatogonia, although a larger dose is needed to effect them in the latter.[62] All of these changes followed acute irradiation. Lorenz and his associates[53] could find no evidence of an effect on the germ plasm when minute doses of gamma rays were applied at low intensity over long periods to successive generations. The special sensitivity of the spermatozoa has stimulated Muller[61] to say that "it should, therefore, be mandatory for a man to abstain from acts of reproduction for some 2 months after his testes have been exposed to any considerable dose of radiation." Likewise the genetic consequences of mass irradiation on large populations have been considered recently by Muller[61] who has stated "if, as in flies, a total dose of 50 r. units, applied to the spermatozoa, results in a mutation frequency about equal to the natural mutation frequency, but added to it, and if this frequency is taken as being about one mutation in 10 to 20 germ cells, then I should hold the effect important." Thus, as the use of atomic energy becomes more and more widespread, the necessity of protecting populations becomes increasingly imperative, even considering the quantitative factors that have been discussed in the preceding paragraphs.

Neoplasia

The existence of a very high incidence of deep burns involving large areas of skin would suggest that carcinoma arising in the epithelium covering the scars will be observed. It will be of interest to determine whether patients who had flash burns, and who consequently were exposed to intense ultraviolet radiation, will have a higher incidence than those whose burns were produced merely by contact with flame or hot objects. Whether the additional factor of exposure to gamma radiation will increase the incidence of carcinoma arising in the burned integument likewise remains to be determined.

Radiation dermatitis, whose precancerous nature has been described by Wolbach,[96] Saunders and Montgomery,[76] and others, was not found

among the survivors. An erythema dose to the skin as a whole would be fatal, especially if the soft rays responsible for the latter were mixed with more penetrating radiations.

Bone sarcomas in man have been described after radiation of benign tumors or even normal bone.[15] Large doses locally applied, however, appear to be required before tumors will result. Such doses again would be fatal if applied to the body as a whole. A much more palpable danger would exist from the ingestion or inhalation of radioactive material, as Martland[55] showed in the radium dial painters. Lisco, Finkel, and Brues[51] already have produced bone tumors experimentally with plutonium and radioactive fission products. In the patients subjected to the atomic bomb there was no evidence of the introduction of radioactive material.

Ovarian tumors have been produced in mice with remarkably small doses of gamma rays—as little as 50 r. given at one time, or 0.11 r. given daily for a total of 90 r.[44,53] The tumors—granulosa cell, tubular adenoma, or luteoma—appear after atrophy of the germ cells. Nothing comparable has as yet been observed in human pathology, but women exposed in the bombed cities should certainly be studied with care over a long period.

The evidence concerning the leukemogenic action of x-rays has been reviewed by Furth.[28] Chronic irradiation has repeatedly been proved to increase the incidence of leukemia in susceptible strains of mice.[40,53] Also, radiologists are known to have a death rate from leukemia greater than that of other physicians.[54] Relatively little is known about the effect of single doses of penetrating gamma rays. Furth concluded that x-rays are only weakly leukemogenic and then only after a long incubation period. There has not been an unduly high incidence of leukemia at Hiroshima and Nagasaki to date as far as can be determined,[91] and the single case of monocytic leukemia is considered purely adventitious. However, further observation will be necessary before this question is decided.

Little material is at hand by which any possible effect on the growing tissue of bone can be evaluated. In clinical experience, radiation possessing certain qualities and locally applied has been found to produce damage to the epiphysis without visible injury to the skin. Whether radiation of the intensity necessary to produce this effect is within the tolerance range when applied to the whole body remains to be determined by biometric and roentgenographic studies of surviving irradiated children of the Japanese cities.

SUMMARY AND CONCLUSIONS

The explosion of the atomic bomb produced mechanical, thermal, and ionizing radiation injuries. The last were quantitatively the least important in the production of casualties.

Direct blast injuries analogous to those inflicted by high explosives were almost unknown among survivors, as indicated by an incidence of ruptured eardrums of about 1 per cent. Almost universal, however, was damage produced by flying glass and the falling beams of wooden houses. The more severe injuries were rare since those that had been severely hurt were killed by fires that swept the city before rescue operations could be instituted.

The burns among survivors were largely of the "flash" type, the result of an exceedingly large quantity of radiant heat acting for an exceedingly brief interval. The effects of exposure extended to approximately 4000 yds. from the bomb. Only survivors in the direct path of the rectilinear rays were involved, so that the burns were of a sharply outlined "profile" or "mask" type. Depigmentation at the center with marginal hyperpigmentation of the burns was prominent in patients close to the bomb, but at greater distances the entire exposed surface became intensely pigmented, and the pigment showed no tendency to fade within 4 months. There was histologic evidence that depigmentation occurred without destruction of the squamous epithelium of the surface, suggesting the action of specific wavelengths.

Even minor injuries and burns became serious foci of infection in persons who also suffered the leukopenia resulting from radiation.

The effects of ionizing radiations resembled closely those produced by total body x-irradiation of animals and men. A special effort was made to center the study of the lesions in patients who had sustained little or no other injury. The effects of ionizing radiations were observed in varying degrees of severity in poorly shielded patients who had been within approximately a mile from the bomb.

Nausea and vomiting occurred in many adequately exposed persons within a few hours after the bomb. The earliest autopsy material was from persons dying "mysteriously" with symptoms of severe diarrhea and fever on the third day after the bomb. In them, epilation and purpura had not had time to appear. After the end of the second week, however, these signs became manifest, and the infective complications of an aplastic anemia became increasingly prominent. Leukopenia had been observed within the first few days after the bombing. After the sixth week, the bone marrow tended to recover and the mortality de-

clined sharply, although an occasional patient succumbed to organizing pneumonitis or ulcerative enteritis. At necropsy, changes were found in the skin, gastro-intestinal tract, gonads, and hemopoietic tissues.

The Skin

In a few cases there were suggestive epithelial changes at the margins of ulcerative lesions in patients dying in the third week, but since most persons who received more than an erythema dose over the whole body died during the first confused days when autopsies were extremely rare, little material was available for study. Epilation in both men and women usually began 14 to 20 days after the bomb. It involved chiefly the scalp in a distribution resembling that of ordinary baldness. Histologically, the mechanism appeared to be entirely analogous to that of the usual processes of loss and replacement of the hair, arrest of mitosis in the matrix, failure of differentiation of the internal root sheath with extrusion of old hair, and finally (some 2 months after irradiation) renewed differentiation of the internal root sheath with penetration of the new hair through the old external sheath to the surface.

Gastro-intestinal Tract

Typical radiation changes were seen in the intestines of persons dying as early as the fourth day. These consisted of the appearance of bizarre cells, some with enormous nuclei possessing a coarse chromatin network and a large body of cytoplasm. Some cells were found in atypical mitosis and tripolar mitotic figures were observed. In one patient who died on the tenth day, the cytoplasm and nuclei of the squamous epithelial cells of the pharynx and tongue were remarkably swollen, and fragmentation of the nuclei was observed.

Gonads

Even at the fourth day remarkable changes were found in the testes, with detachment of the germinal epithelium together with an increase in Sertoli cells. Toward the end of the first month, there was almost complete loss of germinal epithelium. After the fifth week the tubules began to display thickening of the basement membrane and there were hyaline deposits restricting the lumina of the interstitial blood vessels. There was questionable hyperplasia of the interstitial tissue after the end of the sixth week. Clinically, there was a remarkable decrease in the count of spermatozoa of patients who had been close to the bomb. How permanent this will be is at present unknown. "Castration cells" were found occasionally in the pituitary body.

Much less striking changes were observed in the ovary. A few primary follicles were in process of atresia. The most usual finding was that of the absence of developing follicles despite the persistence of primary follicles. The endometrium showed an absence of corpus luteum effect.

Lymphoid Tissues

When first seen, after 3 days, there was a remarkable degree of atrophy of the lymphoid tissue, including those of the spleen, leaving nothing but the reticular skeleton. Beginning on the fifth day, however, large numbers of atypical mononuclear cells resembling lymphoblasts or Reed-Sternberg cells began to appear. These gradually decreased in number during the following 3 months and in a few instances secondary follicles had reappeared by the end of this time both in the spleen and lymph nodes.

Bone Marrow

Even within the first week, in heavily irradiated individuals, almost all mature myeloid and erythroid tissue had disappeared, but there already was evidence of proliferative activity on the part of the reticulum cells. During the first month such proliferative activity became remarkable in many cases, but the products were largely atypical reticulum cells and plasma cells. In some patients, after various lengths of time, there was renewed differentiation into granulopoietic and erythropoietic tissue, and in some, who died toward the end of the sixth week, actual hyperplasia of this tissue was observed, although peripheral leukopenia had been noted.

After the sixth week more and more bone marrows tended to show hyperplasia of myeloid cells and the incidence of leukopenia decreased. In all of these marrows, however, considerable numbers of reticulum cells, plasma cells, and lymphocytes persisted in increased numbers. As the marrow recovered, polymorphonuclear leukocytes became numerous in the lesions and hemorrhage ceased to be an important factor. The important lesions at necropsy at this time were either a necrotizing pneumonia, sometimes in process of organization, or an ulcerative enteritis. Thrombocytopenia was at its lowest level at about the third to fourth week. Thereafter there was rapid recovery. Anemia also was severe during the third to fifth weeks in many patients, but in others it gradually reached its lowest levels thereafter. Recovery in these people was sluggish. This has been associated with dietary deficiency as well as with the effects of the ionizing radiation and of the infections that followed.

No defense has as yet been developed against the destructive effects upon cells subjected to massive amounts of ionizing radiations. Hope

for success in treatment lies in the remarkable resistance of the reticulum cell and the tendency of the marrow ultimately to recover. Pending the resurrection of the marrow, the main therapeutic problems are those of hemorrhage and infection. Despite recent advances, new and more efficient methods for meeting this double challenge remain to be discovered.

Among the many problems that are still to be investigated among the populations of Hiroshima and Nagasaki are: (1) Whether permanent damage of such nature as to reduce the life span was inflicted upon survivors who have apparently recovered. (2) Whether there will be an increased incidence of neoplasia (including leukemia) among the burned or irradiated patients. (3) Whether the growth of irradiated children has been impaired. (4) Whether permanent sterility was induced in any group of survivors. (5) Whether genetic changes will appear, as indicated by decreased fertility, or demonstrable anatomic or physiologic changes. These problems will require many years, or even generations for their solution, if they can be solved at all.

REFERENCES

1. Albers-Schönberg. Ueber eine bisher unbekannte Wirkung der Röntgenstrahlen auf den Organismus der Tiere. *München. med. Wchnschr.*, 1903, 50, 1859–1860.

2. Allen, A. C., and Spitz, S. A comparative study of the pathology of scrub typhus (tsutsugamushi disease) and other rickettsial diseases. *Am. J. Path.*, 1945, 21, 603–681.

3. Allen, J. G., Bogardus, G., Jacobson, L. O., and Spurr, C. L. Some observations on bleeding tendency in thrombocytopenic purpura. *Ann. Int. Med.*, 1947, 27, 382–395.

4. Allen, J. G., and Jacobson, L. O. Hyperheparinemia: Cause of the hemorrhagic syndrome associated with total exposure to ionizing radiation. *Science*, 1947, 105, 388–389.

5. Ashe, W. F., Jr., and Roberts, L. B. Experimental human burns. Partial report. *War Med.*, 1945, **7**, 82–83.

6. Auburtin, G. Das Vorkommen von Kolbenhaaren und die Veränderungen derselben beim Haarwiederersatz. *Arch. f. mikr. Anat.*, 1896, **47**, 472–500.

7. Auburtin, G. Ueber physiologische und pathologische Verschiedenheiten des Haarbodens. Inaugural dissertation. C. Vogt, Berlin, 1895, 27 pp.

8. Barratt, J. O. W., and Arnold, G. Cell changes in the testis due to x-rays. *Arch. f. Zellforsch.*, 1911–12, **7**, 242–276.

9. Block, M. A., and Tsuzuki, M. Observations of burn scars sustained by atomic bomb survivors. *Am. J. Surg.*, 1948, **75**, 417–434.

10. Bloom, W. Histological changes following radiation exposures. *Radiology*, 1947, **49**, 344–347.

11. Bloom, W., and Jacobson, L. O. Some hematologic effects of irradiation. *Blood*, 1948, **3**, 586–592.

12. Blum, H. F. The physiological effects of sunlight on man. *Physiol. Rev.*, 1945, **25**, 483–530.

13. Blum, H. F., and Teris, W. S. Studies of sunburn. Inhibition of erythema by larger doses of ultraviolet radiation. U.S. Naval Medical Research Institute. Research Project X-108, Report No. 3.

14. Brues, A. M., and Jackson, E. B. Nuclear abnormalities resulting from inhibition of mitosis by colchicine and other substances. *Am. J. Cancer*, 1937, **30**, 504–511.

15. Cahan, W. G., Woodard, H. Q., Higinbotham, N. L., Stewart, F. W., and Coley, B. L. Sarcoma arising in irradiated bone. *Cancer*, 1948, **1**, 3–29.

16. Chrom, S. A. Studies on the effect of roentgen rays upon the intestinal epithelium and upon the reticulo-endothelial cells of the liver and spleen. *Acta radiol.*, 1935, **16**, 641–660.

17. Clark, G. L. A 1936 survey of the biological effects of x-radiation. *Radiology*, 1936, **26**, 295–312.

18. Coutard, H. Sur les délais d'apparition et d'évolution des réactions de la peau, et des muqueuses de la bouche et du pharynx, provoquées par les rayons x. *Compt. rend. Soc. de biol.*, 1922, **86**, 1140–1142.

19. Dennis, E. W. Experimental granulopenia, due to bacterial toxins elaborated *in vivo*. *J. Exper. Med.*, 1933, **57**, 993–1008.

20. Draeger, R. H., Barr, J. S., and Sager, W. W. Blast injury. *J. A. M. A.*, 1946, **132**, 762–767.

21. Dunlap, C. E. III. Effects of radiation on the blood and the hemopoietic tissues, including the spleen, the thymus and the lymph nodes. In: Warren, S. Effects of radiation on normal tissues. *Arch. Path.*, 1942, **34**, 562–608.

22. Dunlap, C. E. The effect of roentgen rays and exposure to radium on fertility. *Human Fertil.*, 1946–47, **12**, 33–39.

23. Engelstad, R. B. Histologische Veränderungen in den Nebennieren nach Röntgenbestrahlung. Experimentelle Untersuchungen an Kaninchen. *Strahlentherapie*, 1936, **56**, 58–68.

24. Engelstad, R. B. Über die Wirkungen der Röntgenstrahlen auf die Lungen. *Acta radiol.*, 1934, suppl. 19, 94 pp.

25. Engelstad, R. B., and Torgersen, O. Experimental investigations on the effects of roentgen rays on the suprarenal glands in rabbits. *Acta radiol.*, 1937, **18**, 671–687.

26. Fogg, L. C., and Warren, S. Some cytologic effects of therapeutic irradiation. *Cancer Research*, 1941, **1**, 649–652.

27. Friedman, N. B. IV. Effects of radiation on the gastrointestinal tract, including the salivary glands, the liver and the pancreas. In: Warren, S. Effects of radiation on normal tissues. *Arch. Path.*, 1942, **34**, 749–787.

28. Furth, J. Recent experimental studies on leukemia. *Physiol. Rev.*, 1946, **26**, 47–76.

29. Gates, O. XII. Effects on bone, cartilage and teeth. In: Warren, S. Effects of radiation on normal tissues. *Arch. Path.*, 1943, **35**, 323–340.

30. Goldstein, L., and Murphy, D. P. Etiology of the ill health in children born after maternal pelvic irradiation. II. Defective children born after post-conception pelvic irradiation. *Am. J. Roentgenol.*, 1929, **22**, 322–331.

31. Gore, I., and Saphir, O. Myocarditis associated with acute nasopharyngitis and tonsillitis. *Am. Heart J.,* 1947, **34**, 831–851.

32. Hamilton, J. G. The metabolism of the fission products and the heavier elements. *Radiology*, 1947, **49**, 325–343.

33. Hance, R. T., and Murphy, J. B. Studies on x-ray effects. XV. The prevention of pigment formation in the hair follicles of colored mice with high voltage x-ray. *J. Exper. Med.*, 1926, **44**, 339–342.

34. Heineke, H. Experimentelle Untersuchungen über die Einwirkung der Röntgenstrahlen auf das Knochenmark, nebst einigen Bemerkungen über die Röntgentherapie der Leukämie und Pseudoleukämie und des Sarkoms. *Deutsche Ztschr. f. Chir.*, 1905, **78**, 196–230.

35. Heineke, H. Ueber die Einwirkung der Röntgenstrahlen auf innere Organe. *München. med. Wchnschr.*, 1904, **51**, 785–786.

36. Heineke, H. Ueber die Einwirkung der Röntgenstrahlen auf Tiere. *München. med. Wchnschr.*, 1903, **50**, 2090–2092.

37. Henshaw, P. S. Experimental roentgen injury. I. Effects on the tissues and blood of C₃H mice produced with single small whole-body exposures. *J. Nat. Cancer Inst.*, 1943–44, **4**, 477–484.

38. Henshaw, P. S. Experimental roentgen injury. II. Changes produced with intermediate-range doses and a comparison of the relative susceptibility of different kinds of animals. *J. Nat. Cancer Inst.*, 1943–44, **4**, 485–501.

39. Henshaw, P. S. Experimental roentgen injury. III. Tissue and cellular changes brought about with single massive doses of radiation. *J. Nat. Cancer Inst.*, 1943–44, **4**, 503–512.

40. Henshaw, P. S. Leukemia in mice following exposure to x-rays. *Radiology*, 1944, **43**, 279–285.

41. Henshaw, P. S., Riley, E. F., and Stapleton, G. E. The biologic effects of pile radiations. *Radiology*, 1947, **49**, 349–359.

42. Hickey, P. M., and Hall, E. W. A report analyzing the results of the questionnaire sent out to radiologists, under the direction of the sex committee of the National Research Council. *Am. J. Roentgenol.*, 1927, **18**, 458–462.

43. Huss, J. H., Gilbert, J., and Liebow, A. A. A method for obtaining bone marrow by vertebral spinous process puncture. *Yale J. Biol. & Med.*, 1947–48, **20**, 291–297.

44. Jacobson, L. O., and Marks, E. K. The hematological effects of ionizing radiations in the tolerance range. *Radiology*, 1947, **49**, 286–297.

45. Jacox, H. W. Recovery following human ovarian irradiation. *Radiology*, 1939, **32**, 538–545.

46. Joël, C. A. Veränderungen der Hypophyse nach Röntgenbestrahlung der Hoden. *Schweiz. med. Wchnschr.*, 1942, **23**, 795–796.

47. Joint Commission for the Investigation of the Effects of the Atomic Bomb in Japan. Medical Report. (To be published.)

48. Krumbhaar, E. B. A hematopoietic perifollicular envelope in the rat spleen. *Blood*, 1948, **3**, 953–959.

49. Lawrence, J. H., and Tennant, R. The comparative effects of neutrons and x-rays on the whole body. *J. Exper. Med.*, 1937, **66**, 667–688.

50. Lawrence, J. S., Valentine, W. N., and Dowdy, A. H. The effect of radiation on hemopoiesis. Is there an indirect effect? *Blood*, 1948, 3, 593–611.

51. Lisco, H., Finkel, M. P., and Brues, A. M. Carcinogenic properties of radioactive fission products and of plutonium. *Radiology*, 1947, 49, 361–362.

52. Loge, J. P. Spinous process puncture. A simple clinical approach for obtaining bone marrow. *Blood*, 1948, 3, 198–204.

53. Lorenz, E., Heston, W. E., Eschenbrenner, A. B., and Deringer, M. K. Biological studies in the tolerance range. *Radiology*, 1947, 49, 274–285.

54. March, H. C. Leukemia in radiologists. *Radiology*, 1944, 43, 275–278.

55. Martland, H. S. The occurrence of malignancy in radioactive persons. A general review of data gathered in the study of the radium dial painters, with special reference to the occurrence of osteogenic sarcoma and the interrelationship of certain blood diseases. *Am. J. Cancer*, 1931, 15, 2435–2516.

56. Masui, K., and Tamura, Y. The effect of gonadectomy on the structure of the suprarenal gland of mice. *J. Coll. Agri., Imper. Univ. Tokyo*, 1926, 7, 353. (Cited by Moore, C. R. Biology of the Testes. In: Allen, E. (ed.) Sex and Internal Secretions. Williams & Wilkins Co., Baltimore, 1932, p. 338.

57. Maximow, A. A. Studies on the changes produced by roentgen rays in inflamed connective tissue. *J. Exper. Med.*, 1923, 37, 319–340.

58. Mayer, M. D., Harris, W., and Wimpfheimer, S. Therapeutic abortion by means of x-ray. *Am. J. Obst. & Gynec.*, 1936, 32, 945–957.

59. Minot, G. R., and Spurling, R. G. The effect on the blood of irradiation, especially short wave length roentgen-ray therapy. *Am. J. M. Sc.*, 1924, 168, 215–241.

60. Moon, V. H., Kornblum, K., and Morgan, D. R. The nature and pathology of radiation sickness. *J. A. M. A.*, 1941, 116, 489–493.

61. Muller, H. J. Mutational prophylaxis. *Bull. New York Acad. Med.*, 1948, 24, s.2, 447–469.

62. Muller, H. J. The Effects of Roentgen Rays upon the Hereditary Material. In: Glasser, O. The Science of Radiology. Charles C Thomas, Springfield, Ill., 1933, pp. 305–318.

63. Murphy, D. P. The outcome of 625 pregnancies in women subjected to pelvic radium or roentgen irradiation. *Am. J. Obst. & Gynec.*, 1929, 18, 179–187.

64. Murphy, D. P., and Goldstein, L. Etiology of the ill-health of children born after maternal pelvic irradiation. Part I. Unhealthy children born after preconception pelvic irradiation. *Am. J. Roentgenol.*, 1929, 22, 207–219.

65. Naujoks, H. Fertilität und Nachkommenschaft früherer Röntgenassistentinnen. *Strahlentherapie*, 1929, 32, 613–639.

66. Oliver, J., Bloom, F., and Mangieri, C. On the origin of heparin. *J. Exper. Med.*, 1947, 86, 107–116.

67. Peck, W. S., McGreer, J. T., Kretzschmar, N. R., and Brown, W. E. Castration of the female by irradiation. The results in 334 patients. *Radiology*, 1940, 34, 176–186.

68. Pinkus, F. Die normale Anatomie der Haut. Der Haarwechsel. In: Jadassohn, J. Handbuch der Haut- und Geschlechtskrankheiten. Julius Springer, Berlin, 1927, 1, Pt. 1, 212–217.

69. Prosser, C. L. The clinical sequence of physiological effects of ionizing radiation in animals. *Radiology*, 1947, 49, 299–312.

70. Raper, J. R. Effects of total surface beta irradiation. *Radiology*, 1947, 49, 314–324.

71. Rhoads, C. P., and Miller, D. K. Histology of the bone marrow in aplastic anemia. *Arch. Path.*, 1938, **26**, 648–663.

72. Rich, A. R., Lewis, M. R., and Wintrobe, M. M. The activity of the lymphocyte in the body's reaction to foreign protein, as established by the identification of the acute splenic tumor cell. *Bull. Johns Hopkins Hosp.*, 1939, **65**, 311–326.

73. Rock, J., Bartlett, M. K., Gauld, A. G., and Rutherford, R. N. The effect of subcastrative roentgen therapy on ovarian physiology. *Surg., Gynec. & Obst.*, 1940, **70**, 903–913.

74. Rogers, F. T., and Martin, C. L. The effects of x-rays on the adrenal gland. *Am. J. Physiol.*, 1930, **93**, 219–226.

75. Rohr, K. Das menschliche Knochenmark: Seine Anatomie, Physiologie und Pathologie nach Ergebnissen der intravitalen Sternalpunktion. Georg Thieme, Leipzig, 1940, 286 pp.

76. Saunders, T. S., and Montgomery, H. Chronic roentgen and radium dermatitis; an analysis of 259 cases. *J. A. M. A.*, 1938, **110**, 23–28.

77. Severinghaus, A. E. The Cytology of the Pituitary Gland. In: Association for Research in Nervous and Mental Disease, Vol. 17. The Pituitary Gland. Williams & Wilkins Co., Baltimore, 1938, pp. 92–94.

78. Snell, G. D. Induction by roentgen rays of hereditary changes in mice. *Radiology*, 1941, **36**, 189–196.

79. Spear, F. G. The biological effects of penetrating radiations. In: Radiology, experimental and applied. *Brit. M. Bull.*, 1946, **4**, 2–11.

80. Tennant, R., and Liebow, A. A. The actions of colchicine and of ethylcarbylamine on tissue cultures. *Yale J. Biol. & Med.*, 1940, **13**, 39–49.

81. Torgersen, O. Histological studies on the normal and the irradiated suprarenal gland in rabbits. *Skrifter u. a. Norske Videnskaps-Akad. Oslo, I. Mat.-Naturv. Klasse*, 1940, No. 2, 112 pp.

82. Trump, J. G. Physical basis for the high skin tolerance of supervoltage roentgen rays. *Radiology*, 1948, **50**, 649–656.

83. Tsuzuki, M. Experimental studies on the biological action of hard roentgen rays. *Am. J. Roentgenol.*, 1926, **16**, 134–150.

84. Tullis, J. L., and Warren, S. Gross autopsy observations in the animals exposed at Bikini. *J. A. M. A.*, 1947, **134**, 1155–1158.

85. Van Wagenen, G. Histological changes in the male rat hypophysis following degeneration of the germinal epithelium. *Anat. Rec.*, 1924–25, **29**, 398–399.

86. von Wattenwyl, H. Ueber die Wirkung der Röntgenstrahlen auf den Rattenhoden. *Schweiz. med. Wchnschr.*, 1942, **23**, 765–766.

87. Warren, S. Effects of radiation on normal tissues. I. Introduction. II. The effects of radiation on the cell. *Arch. Path.*, 1942, **34**, 443–450.

88. Warren, S. Effects of radiation on normal tissues. VIII. Effects on the gonads. *Arch. Path.*, 1943, **35**, 121–127.

89. Warren, S. Effects of radiation on normal tissues. XIII. Effects on the skin. *Arch. Path.*, 1943, **35**, 340–347.

90. Warren, S. The histopathology of radiation lesions. *Physiol. Rev.*, 1944, **24**, 225–238.

91. Warren, S. The Nagasaki survivors as seen in 1947. *Mil. Surgeon*, 1948, **102**, 98–100.

92. Warren, S., and Gates, O. Radiation pneumonitis. Experimental and pathologic observations. *Arch. Path.*, 1940, **30**, 440–460.

93. Warren, S. L., and Whipple, G. H. Roentgen ray intoxication. I. Bacterial invasion of the blood stream as influenced by x-ray destruction of the mucosal epithelium of the small intestine. *J. Exper. Med.*, 1923, **38**, 713–723.

94. Wigoder, S. B. The effects of x-rays on the testis. *Brit. J. Radiol.*, 1929, **2**, 213–221.

95. Wilder, H. C. Pathology of the eye in atomic bomb casualties. (To be published.)

96. Wolbach, S. B. The pathological histology of chronic x-ray dermatitis and early x-ray carcinoma. *J. M. Research*, 1909, **21**, 415–449.

97. Zirkle, R. E. Components of the acute lethal action of slow neutrons. *Radiology*, 1947, **49**, 271–273.

98. Zuckerman, S. Discussion on the problem of blast injuries. *Proc. Roy. Soc. Med.*, 1940–41, **34**, 171–188.

DESCRIPTION OF PLATES

PLATE 121

FIG. 1. Multiple injuries by flying glass. Patient was in a standing position, approximately 5 ft. from a window, indoors in a military barracks. Upper torso was nude but he was wearing trousers, which were not penetrated by the glass. Keloidal overgrowth of connective tissue. White blood cell count was 13,000 on the 44th day. Takatomi. Male, age unknown. Approximately 1000 yds. Died on the 57th day. Army Institute of Pathology negative no. HP 151A. Bunka-Sha photograph.

FIG. 2. "Flash burns." The darker portions of a striped pattern of cloth that the patient was wearing absorbed more heat and produced the gridiron burns of the skin. The arm below the sleeve line and the unprotected face were most severely burned. The burns are very sharply outlined. Ushio. Female, age unknown. Distance unknown. Late August, 1945, approximately 3 weeks after bombing. A.I.P. neg. HP 138-d. Photo made by Japanese medical officers of Tokyo 1st Military Hospital.

FIG. 3. "Flash burns" of third degree. No protection by the hair. Partial protection of the lower extremities by cloth trousers, and complete protection of skin of abdomen by the multiple layers of a cummerbund. Burns of the back, where there was no clothing, are sharply outlined. Name unknown. Male, age unknown. Distance unknown. A.I.P. neg. HP 142. Photo made by Japanese medical officers of Tokyo 1st Military Hospital.

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PLATE 122

FIG. 4. "Flash burns." Depigmentation sharply outlined by pigmented tissue in a very narrow band. The line of the burn extends upwards on the chin. The darker material in the peri-aural region is not pigmented tissue, but is a crusted exudate and keratin. Kosugi. Female, 17 years of age. Approximately 1700 yds. 100th day. A.I.P. neg. HP 117 (K).

FIG. 5. "Flash burns." Deep chocolate-brown pigmentation. Very sharp outlines. Protection of upper portion of neck by shadow of mandible, and of nasolabial and lateral nasal grooves by the alae and nose. Enami. Male, 30 years of age. Approximately 2300 yds. 99th day. A.I.P. neg. HP 112b (K).

FIG. 6. Flash burns of skin. General view. Zone of greatest destruction of epithelium at the right. Depigmented tissue with relatively well preserved epithelium in central zone. Hyperpigmented tissue at left (see Figs. 11 and 12). K-4. Kume. Male, 32 years of age. Approximately 1000 yds. Died on the sixth day. A.I.P. neg. HM 134 (K). × 15.

FIG. 7. Keloids following flash burns. Protective effect of shoulder straps of slip and of sleeve seam. Pigmentation at margins of the burns. Akamatsu. Female, 21 years of age. Approximately 1400 yds. Approximately 2½ months. A.I.P. neg. HP 111-b (K).
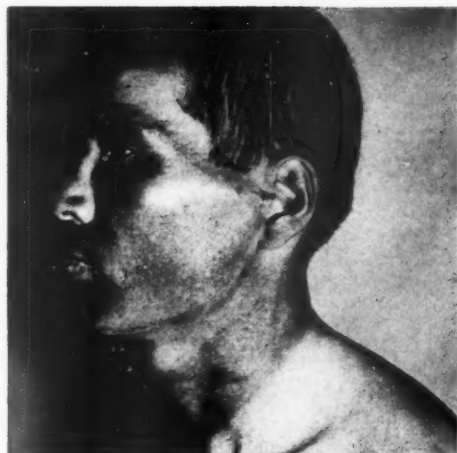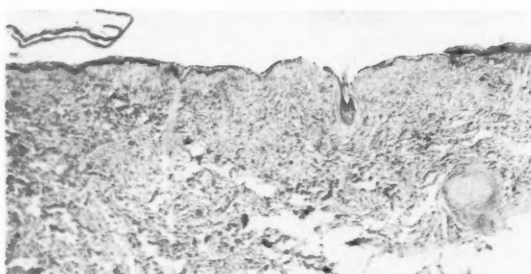
Eebow,

Fɪɢ. 8. "Flash burns" of shoulder. Mottled depigmentation of central portions where burn was most severe, outlined by a band of hyperpigmented tissue. There is a narrow zone of depigmentation between the normal and burned skin. Maeda. Male, 40 years of age. Approximately 2300 yds. 99th day. A.I.P. neg. HP 115-a (K).

Fɪɢ. 9. Epilation of scalp. Scattered long hairs of the original growth remain. Patient was inside a wooden building at Nagasaki at the time of the bombing. Epilation began on the 19th day, 3 days after the appearance of purpura. Leukopenia persisted for 2 months, but the patient recovered. 63rd day. A.I.P. neg. NP 159.

Fɪɢ. 10. Group II. Petechiae of epicardium. K-28. Kawaura. Male, 23 years of age. Approximately 1000 yds. Died on the 26th day. A.I.P. neg. HS 307 (K).
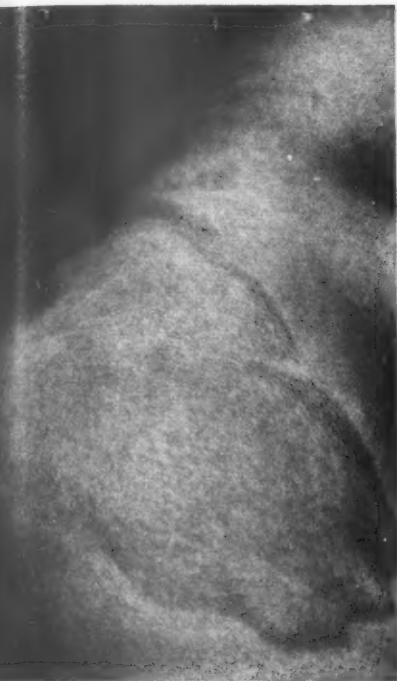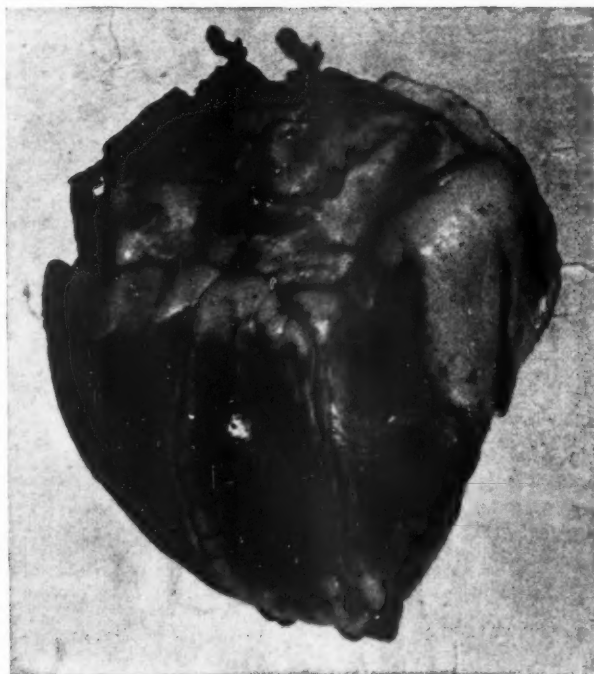
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Fig. 11. Group I. Enlargement of right-hand portion of Figure 6. At right, necrosis of epithelium; infiltration with large mononuclear elements, which also are in process of necrosis. At left, well preserved epithelium without pigment. Dermal melanophores also are not seen here. A.I.P. neg. HM 133. × 100.

Fig. 12. Group I. Enlargement of left-hand margin of Figure 6. Hyperpigmentation of skin. Large numbers of stellate melanophores among the epithelial cells. A.I.P. neg. HM 132. × 200.
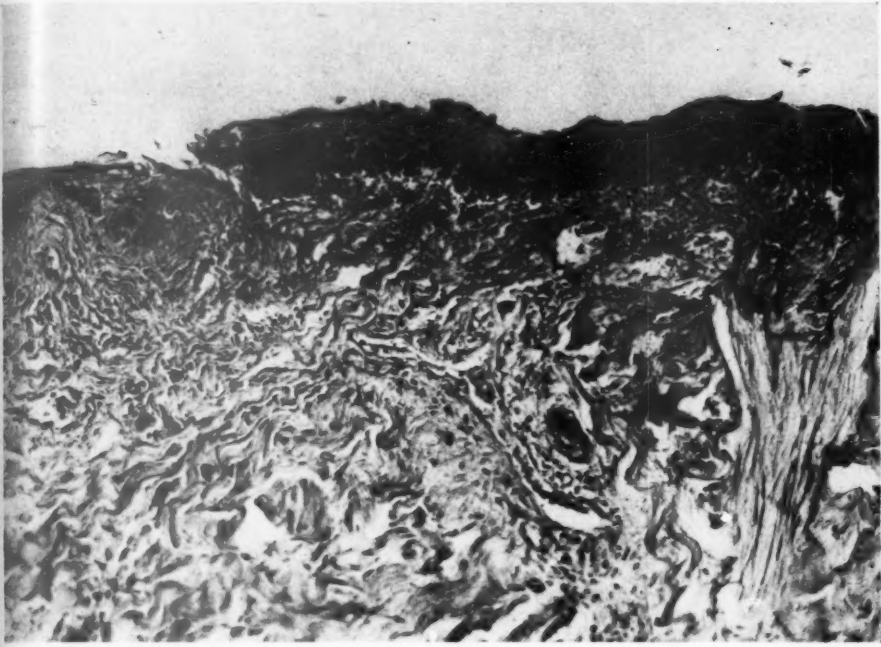
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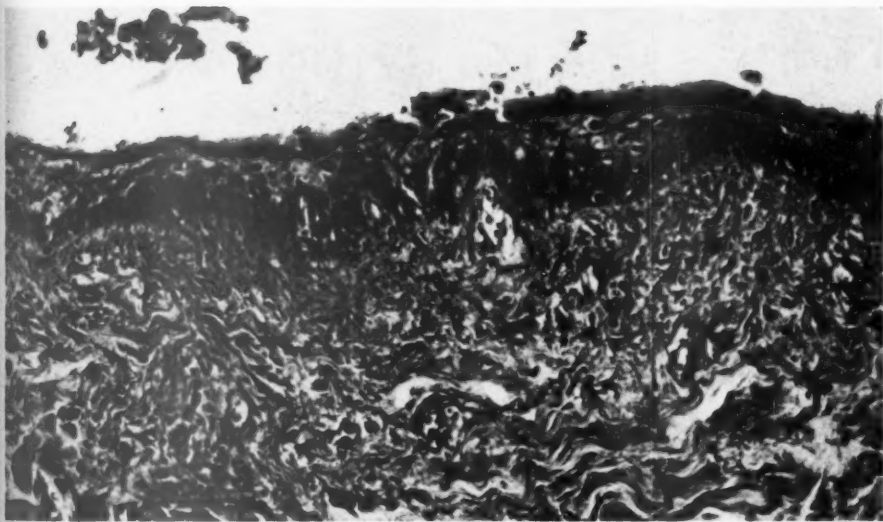
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PLATE 125

FIG. 13. Group I. Skin from burned area. Necrosis of epithelium and outer layers of derma. Bacterial masses in derma without cellular reaction. Vacuolation and shrinkage of acinar epithelium of sweat glands. Squamous metaplasia of duct of sweat gland. Bacterial mass within arrector pili muscle. K-5. Yano. Male, 39 years of age. Approximately 1000 yds. Died on the sixth day. A.I.P. neg. HM 130. × 50.

FIG. 14. Group I. Skin from burned area. Minute thrombus in blood vessel where wall has undergone necrosis. The exudate is almost exclusively of large mononuclear cells. K-3. Sakuma. Male, 15 years of age. Approximately 1000 yds. Died on the fifth day. A.I.P. neg. HM 113. × 350.

FIG. 15. Group I. Subcutaneous tissue deep to burn. Edema and myxomatous change of connective tissue. Proliferation and swelling of histiocytic and fibroblastic elements. Many mast cells are seen. From the same patient as Figure 14. A.I.P. neg. HM 118. × 100.

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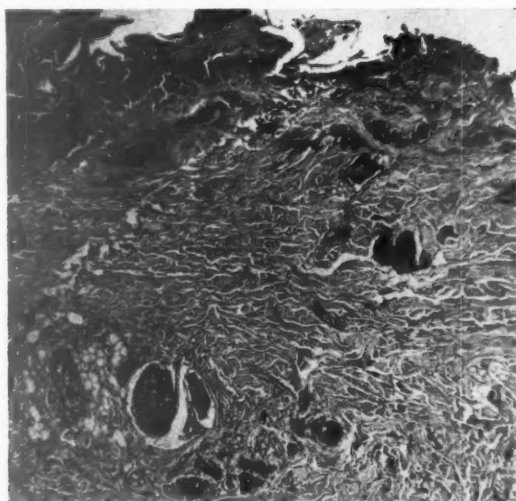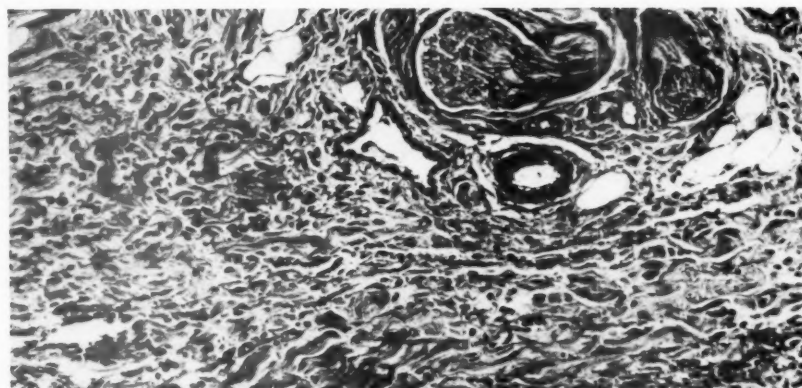
PLATE 126

FIG. 16. Group II. Subendocardial hemorrhages of left ventricle in region of conduction bundle. K-43. Horinouchi. Male, 33 years of age. Approximately 1000 yds. Died on the 33rd day. A.I.P. neg. HS 326b.

FIG. 17. Group II. Focal necrotizing bronchiolitis and focal necrotizing neutropenic pneumonia with surrounding hemorrhage. Relatively more necrosis than in Figure 19. K-36. Morita. Female, 21 years of age. Approximately 1000 yds. Died on the 28th day. A.I.P. neg. HS 319.

FIG. 18. Group II. Lung. Scar of apex. Disseminated caseous tubercles surrounded by hemorrhagic parenchyma. K-40. Motoyama. Male, 29 years of age. Approximately 1000 yds. Died on the 30th day. A.I.P. neg. HS 322.

FIG. 19. Group II. Lung. Focal necrotizing bronchiolitis and focal pneumonia with surrounding hemorrhage. The foci of necrosis are minute. K-44. Araki. Male, 22 years of age. Approximately 1000 yds. Died on the 33rd day. A.I.P. neg. HS 329.
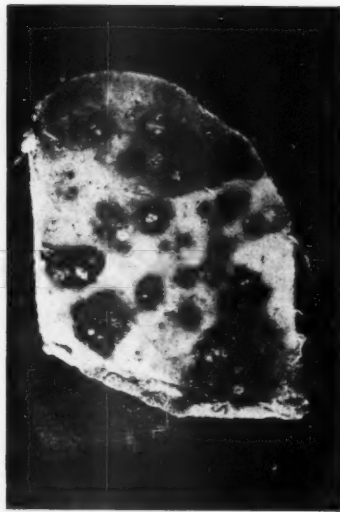
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PLATE 127

FIG. 20. Group I. Lung. Focal emphysema and atelectasis. Edema of pleura. K-1. Harada. Male, 13 years of age. Approximately 1300 yds. Died on the third day. A.I.P. neg. HM 104.

FIG. 21. Group II. Heart. Perivascular and interstitial infiltration with small and large mononuclear cells and plasma cells. K-29. Murakami. Male, 22 or 24 years of age (variously stated). Approximately 1000 yds. Died on the 27th day. A.I.P. neg. HM 154. × 220.
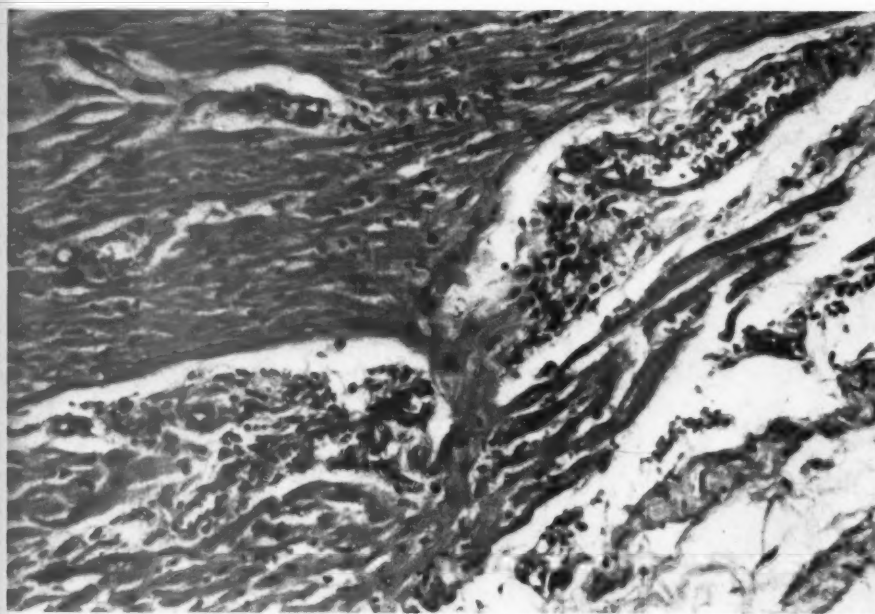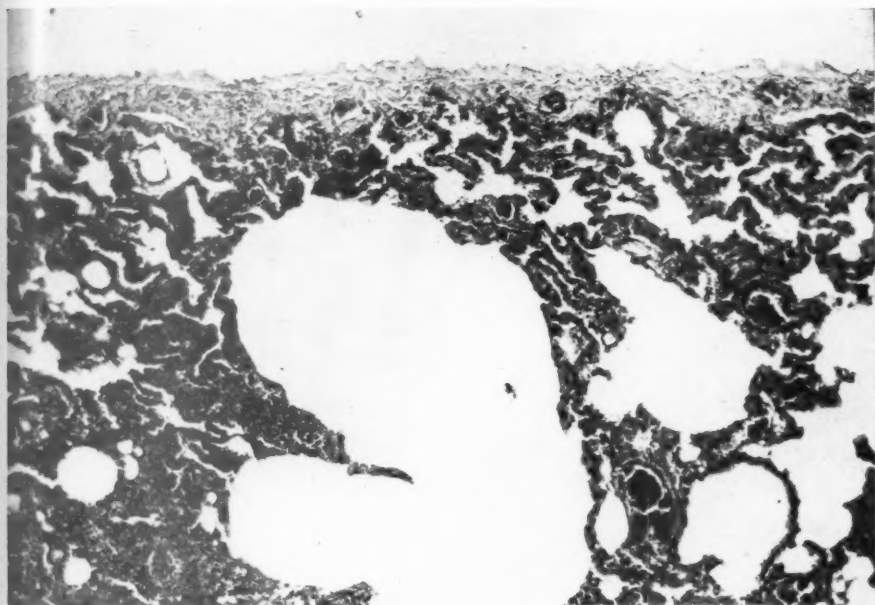
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PLATE 128

FIG. 22. Group II. Lung. Necrosis of bronchiole. Bacterial masses attached to wall. Necrotizing and hemorrhagic pneumonia. K-21. Iseoka. Male, 45 years of age. Approximately 1000 yds. Died on the 24th day. A.I.P. neg. HM 145. ✕ 100.

FIG. 23. Group II. Lung. Focus of "necrotizing and hemorrhagic aplastic pneumonia." Bacterial masses near center of the lesion. Lining membrane of bronchiole near right center of lesion is completely necrotic. K-47. Naka. Female, 35 years of age. Approximately 800 yds. Died on the 18th day. A.I.P. neg. HM 242. ✕ 50.

FIG. 24. Group II. Lung. Edema of pleura. Necrotizing and hemorrhagic "aplastic pneumonia." K-119. Nagado. Female, 26 years of age. Approximately 1300 yds. Died on the 23rd day. A.I.P. neg. HM 288. ✕ 115.
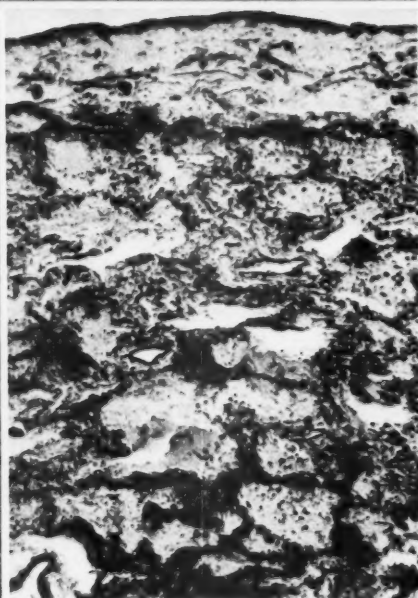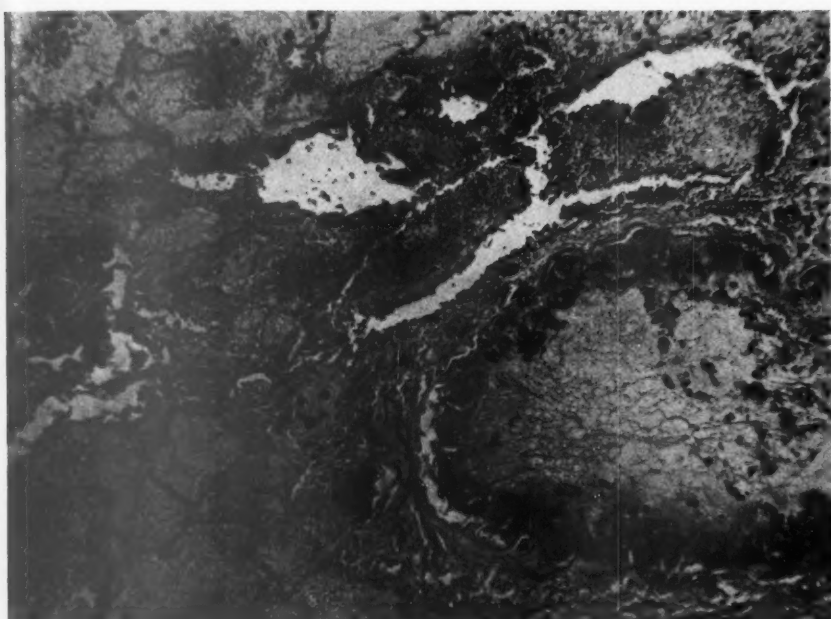
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FIG. 25. Group III. Fibrous wall of abscess cavity. From the same patient as Figure 27. A.I.P. neg. HM 269. × 50.

FIG. 26. Group III. Lung. Organizing pneumonia. K-96. Sakoda. Male, 33 years of age. Approximately 1000 yds. Died on the 97th day. A.I.P. neg. HM 278. × 130.

FIG. 27. Group III. Right lung. Gangrene of upper lobe with sequestrated lung tissue. Abscess cavity in lower lobe. Diffuse necrotizing and organizing pneumonia. K-50. Kijima. Male, 31 years of age. Approximately 1000 yds. Died on the 100th day. A.I.P. neg. HS 333.
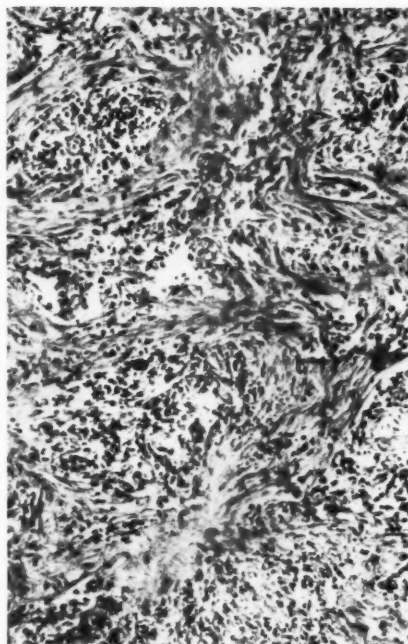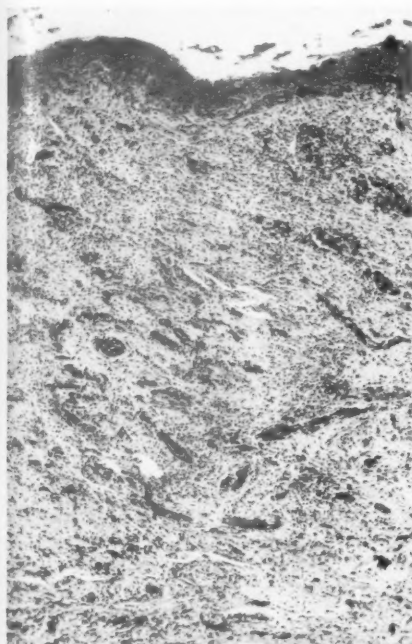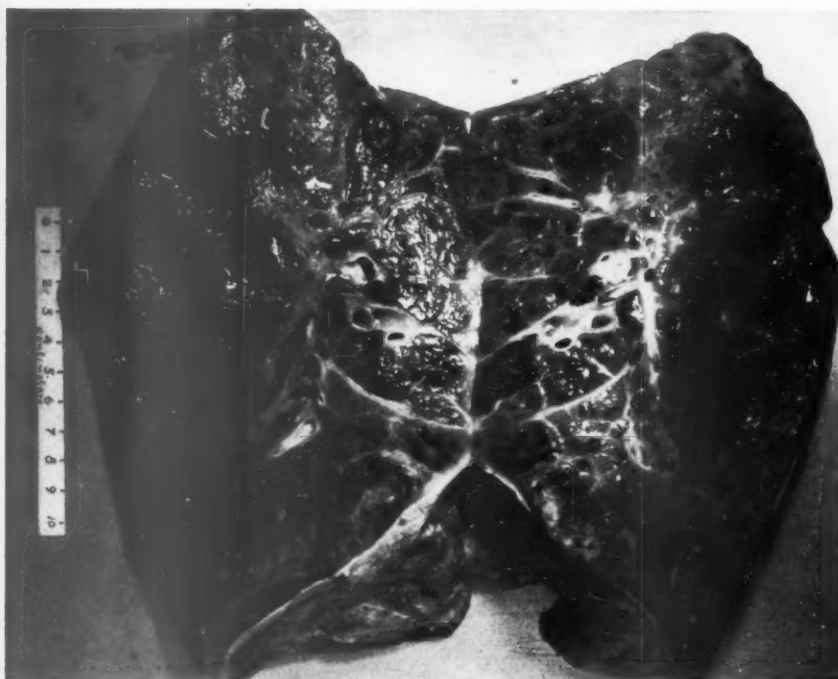
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PLATE 130

FIG. 28. Group I. Spleen. Marked shrinkage of malpighian body. Disappearance of lymphocytes and of cells of Billroth's cords. Acidophilic refractile material in subendothelial position of central vessel. K-2. Onishi. Male, 24 years of age. Approximately 800 yds. Died on the fourth day. A.I.P. neg. HM 102. × 100.

FIG. 29. Group I. Spleen. Disappearance of lymphocytes from sites of malpighian corpuscles. Necrosis *in situ* of lymphocytes and of cells of germinal center. Acidophilic refractile material in subendothelial tissues of central arteries. From the same patient as Figure 28. A.I.P. neg. HM 100. × 400.
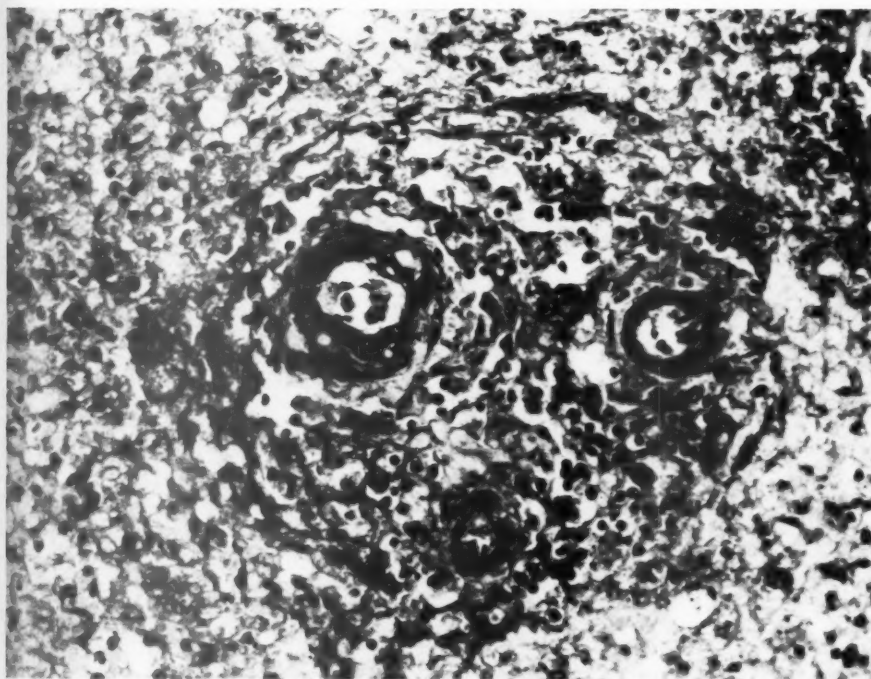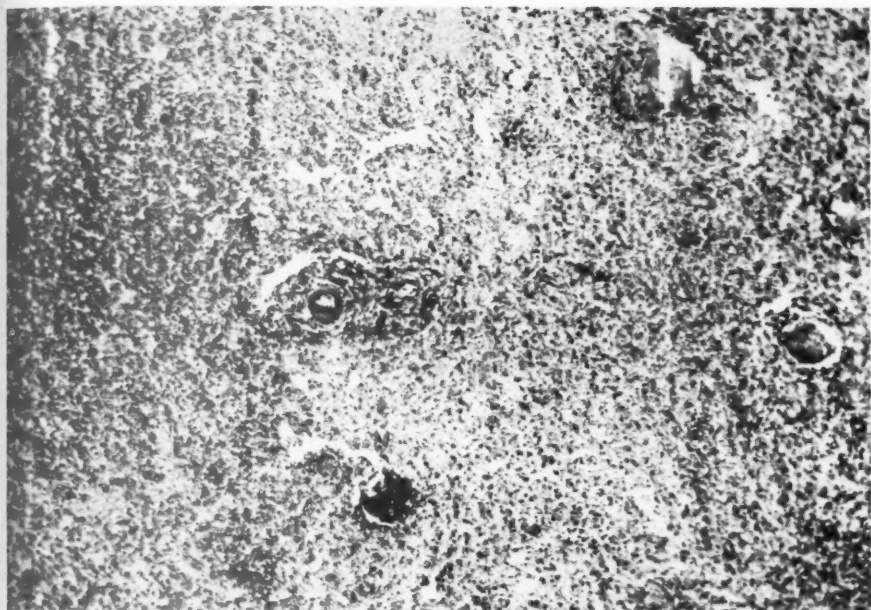
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FIG. 30. Group I. Spleen. Malpighian corpuscle. Paucity of lymphocytes. Giant cell resembling Reed-Sternberg cell. Hemorrhage in and about malpighian follicle. K-3. Sakuma. Male, 15 years of age. Approximately 1000 yds. Died on the fifth day. A.I.P. neg. HM 107. × 165.

FIG. 31. Group I. Spleen. Erythrophagocytosis. Atypical large mononuclear cells. From the same patient as Figure 30. A.I.P. neg. HM 120. × 750.
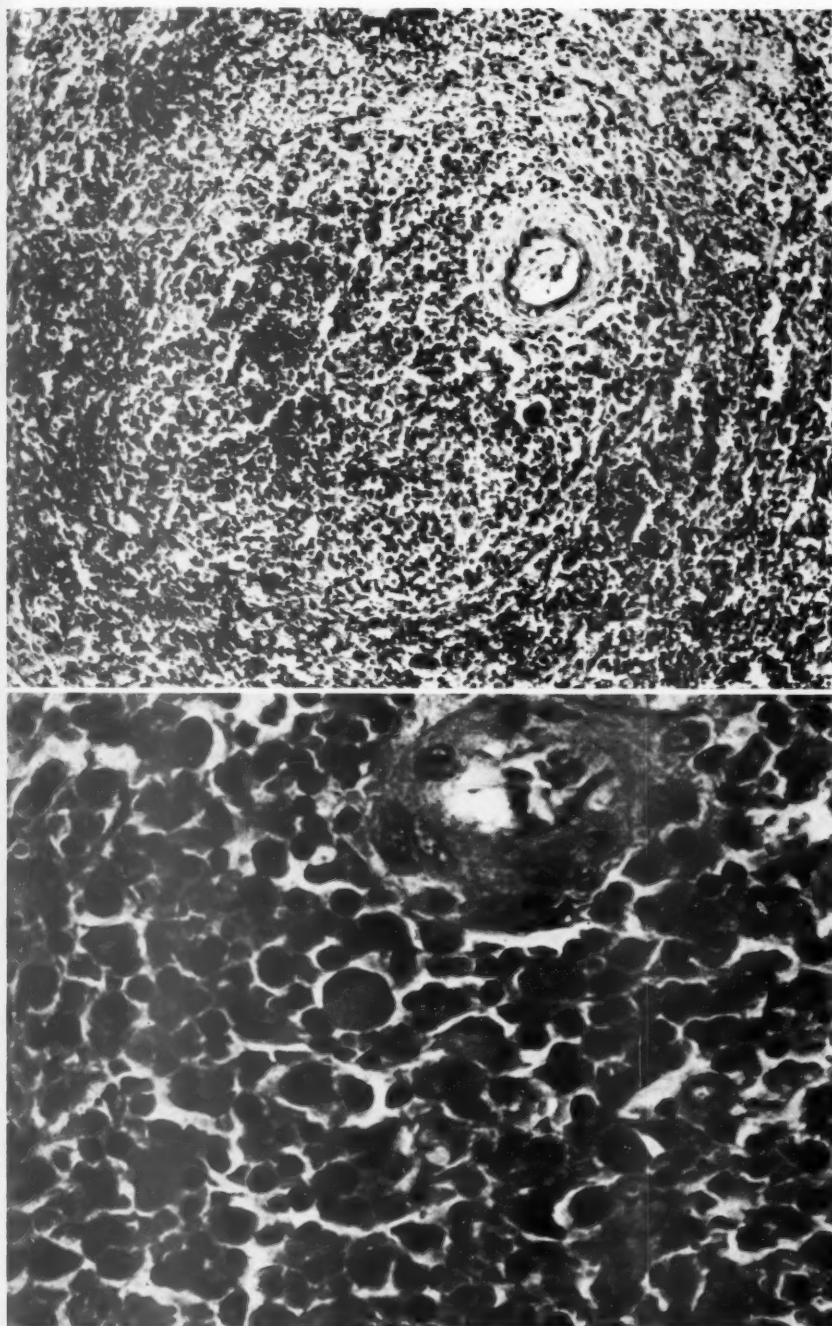
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PLATE 132

FIG. 32. Group I. Spleen. Knot of reticulum cells at center. Large atypical mononuclear elements. Group of minute nuclei. From the same patient as Figure 30. A.I.P. neg. HM 121 (K). × 350.

FIG. 33. Group II. Lymph node. Almost total disappearance of lymphocytes. Skeleton of reticular cells. Numerous mast cells. Giemsa's stain. K-28. Kawaura. Male, 23 years of age. Approximately 1000 yds. Died on the 26th day. A.I.P. neg. HM 295. × 500.

FIG. 34. Group III. Femur. Red gelatinous and gray-red cellular marrow in upper portion of shaft. Gelatinous and fatty yellow marrow in distal portion of the bone. From the same patient as Figure 70. A.I.P. neg. HS 334 (K).

FIG. 35. Group I. Marrow from long bone. Reticulum cells, plasmacytoid elements, and some large cells intermediate in appearance between the two. No normal hemopoietic tissue. Erythrophagocytosis by some of the reticular elements. This is the earliest histologic specimen of bone marrow available. By the criteria employed in the text it would be classified "type A," marked hypoplasia. K-5. Yano. Male, 39 years of age. Approximately 1000 yds. Died on the sixth day. A.I.P. neg. HM 131. × 650.
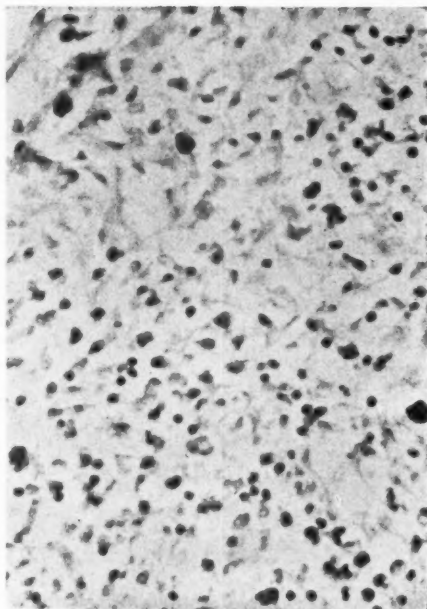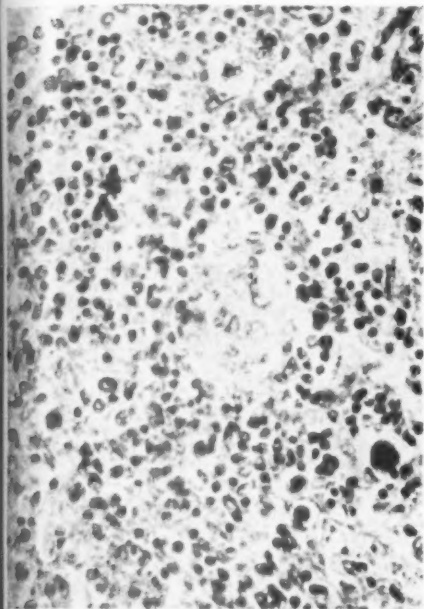
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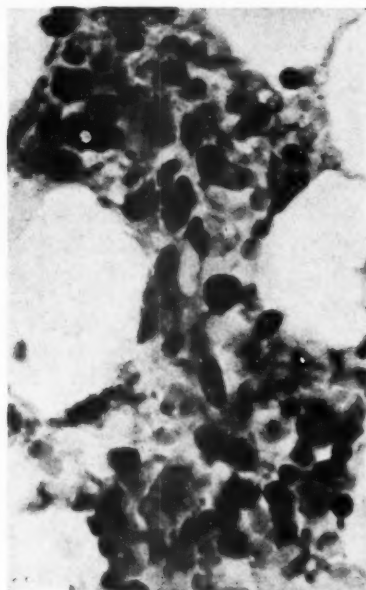
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PLATE 133

FIG. 36. Group II. Smear of bone marrow. Reticulum cells, myelocytes with azurophilic granules, and cells transitional between the two. These transitional forms suggest a direct origin of myelocytes from reticulum cells in these rapidly regenerating marrows. Numerous plasma cells and forms transitional between them and the reticulum cells. Blast cells are rare in this smear. Wright-Giemsa stain. K-43. Horinouchi. Male, 33 years of age. Approximately 1000 yds. Died on the 32nd day. A.I.P. neg. HM 320 (K). × 800.

FIG. 37. Group II. Pituitary body. Vacuolated large basophilic cells ("castration cells"). Hyperplasia of basophilic cells. K-42. Moriseko. Male, 27 years of age. Approximately 1000 yds. Died on the 31st day. A.I.P. neg. HM 298. × 400.

FIG. 38. Group III. Scalp. Atrophy of hair follicles and associated sebaceous glands. There is no notable change in the sweat glands. For details see Figure 141. K-14. Yamamoto. Male, 25 years of age. Approximately 1000 yds. Died on the 47th day. A.I.P. neg. HM 280 (K). × 22.

FIG. 39. Group III. Testis. Disappearance of germinal epithelium and its derivatives. Thickening of basement membranes of the tubules by deposition of finely fibrillar collagenous material within the old basement membrane. Deposits of refractile acidophilic material beneath the endothelium of the blood vessels in the interstitium. K-14. Yamamoto. Male, 25 years of age. Approximately 1000 yds. Died on the 47th day. A.I.P. neg. HM 211. × 115.

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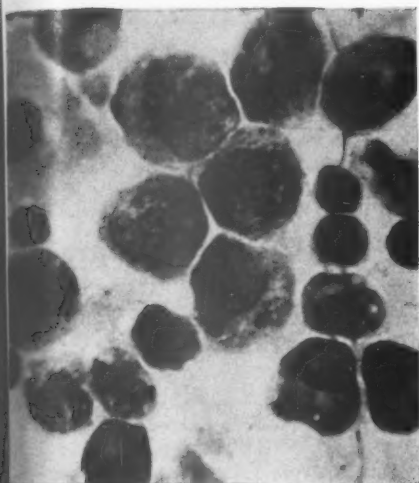
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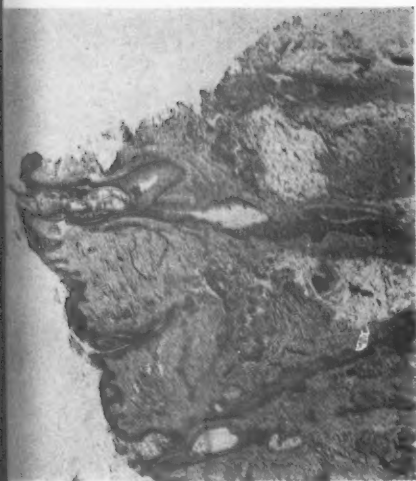
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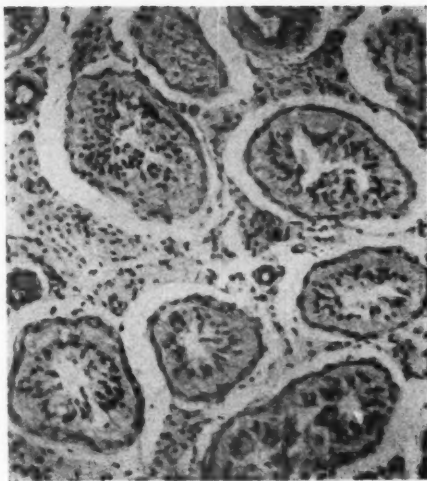
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PLATE 134

FIG. 40. Group I. Spleen. Tissue in neighborhood of central arteriole. Only rare lymphocytes are present. Numerous large atypical mononuclear elements, some in mitosis. K-9. Sakamoto. Male, 25 years of age. Approximately 1500 yds. Died on the eighth day. A.I.P. neg. HM 138. × 400.

FIG. 41. Group II. Spleen. Condensation of reticulum cells about central arteries of malpighian corpuscles. K-35. Takahashi. Male, 31 years of age. Approximately 1000 yds. Died on the 28th day. A.I.P. neg. HM 223. × 120.

FIG. 42. Group II. Spleen. Malpighian corpuscle. Almost total disappearance of typical lymphocytes. A few small and large plasmacytoid elements remain. Deposition of hyaline, homogeneous, acidophilic, refractile material beneath endothelium of central arteriole. Slight, if any, evidence of proliferative activity of the reticulum cells. K-47. Naka. Female, 35 years of age. Approximately 800 yds. Died on the 18th day. A.I.P. neg. HM 247. × 135.

FIG. 43. Group II. Spleen. Atrophy of lymphoid tissue. Atypical large mononuclear elements. Hyaline changes in walls of central arteries. K-121. Sakanishi. Male, 45 years of age. Approximately 1000 yds. Died on the 24th day. A.I.P. neg. HM 306. × 400.

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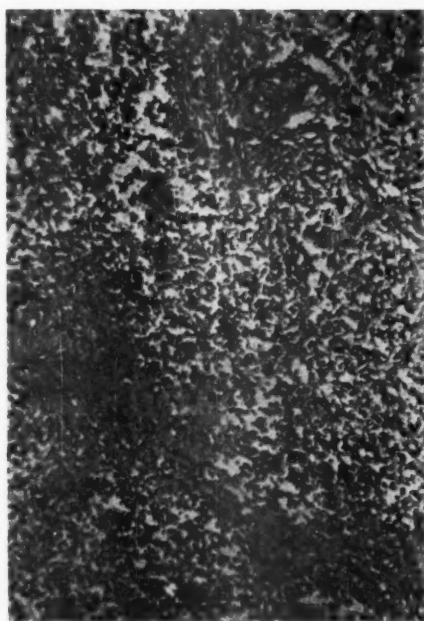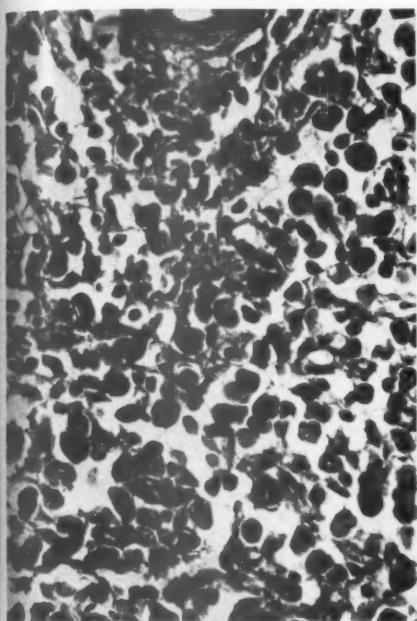
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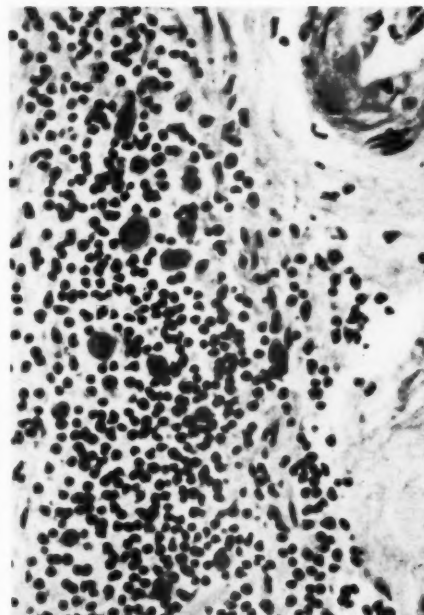
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FIG. 44. Group I. Lymph node. Collapsed reticular skeleton supporting a very few mature lymphocytes. K-98. Tamai. Male, 19 years of age. Distance unknown. Died on the tenth day. A.I.P. neg. HM 282. × 160.

FIG. 45. Group II. Spleen. Condensation of syncytial masses of reticulum cells about a regenerating malpighian corpuscle. Giemsa's stain. From the same patient as Figure 41. A.I.P. neg. HM 224. × 235.

FIG. 46. Group II. Spleen. Condensation of syncytial reticulum cells at margin of regenerating malpighian corpuscle. Giemsa's stain. Enlargement of a portion of Figure 45. A.I.P. neg. HM 225. × 400.
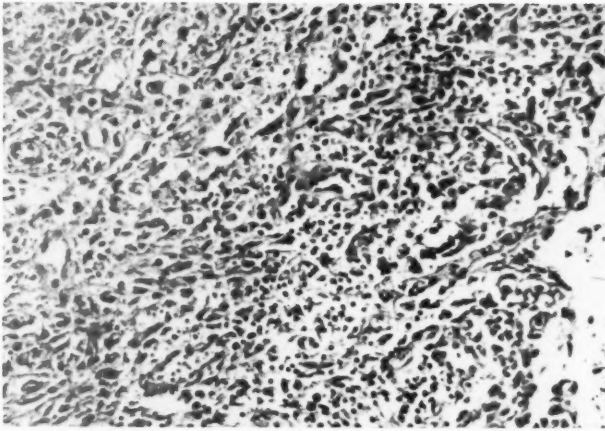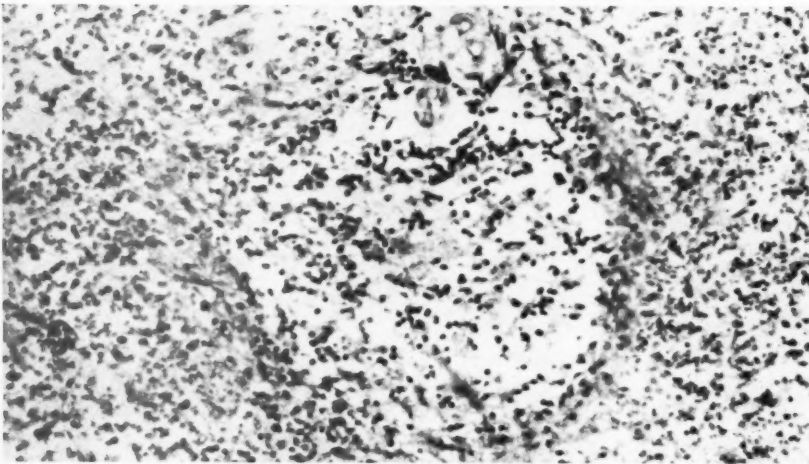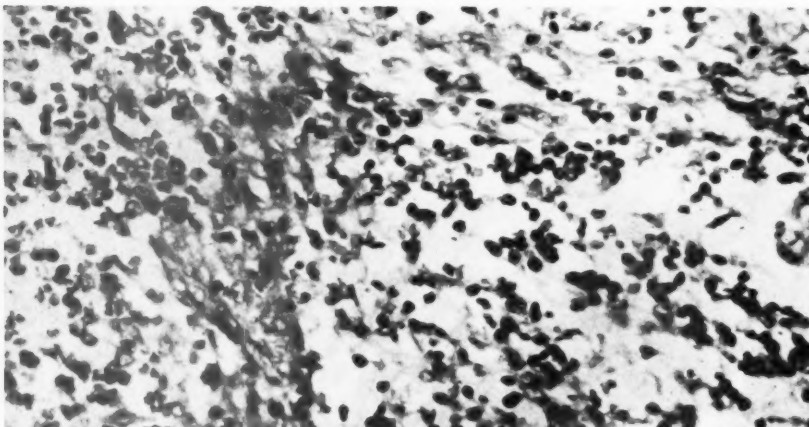
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FIG. 47. Group II. Spleen. Condensation of syncytial reticulum at periphery of regenerating malpighian corpuscle. Beginnings of germinal center. K-38. Kamihara. Male, 22 years of age. Approximately 1000 yds. Died on the 29th day. A.I.P. neg. HM 226. × 145.

FIG. 48. Group III. Spleen. Germinal center in malpighian corpuscle. K-50. Kijima. Male, 31 years of age. Approximately 1000 yds. Died on the 100th day. A.I.P. neg. HM 267. × 200.

FIG. 49. Group II. Lymph node. Absence of germinal centers. Mature small lymphocytes are few. Numerous large lymphocytes, cells with the structure of lymphoblasts, and some elements intermediate in appearance between these and the elements of the reticulum. K-104. Yoshitomi. Male, 14 yrs. of age. Approximately 1000 yds. Died on the 21st day. A.I.P. neg. HM 286. × 115.

FIG. 50. Group II. Lymph node. Enlargement of a portion of Figure 49. A.I.P. neg. HM 285. × 400.
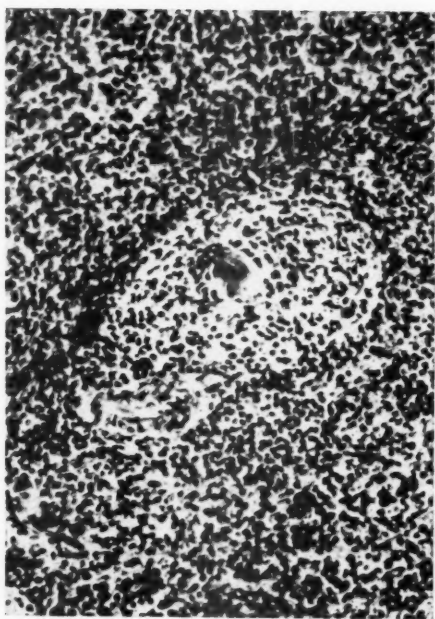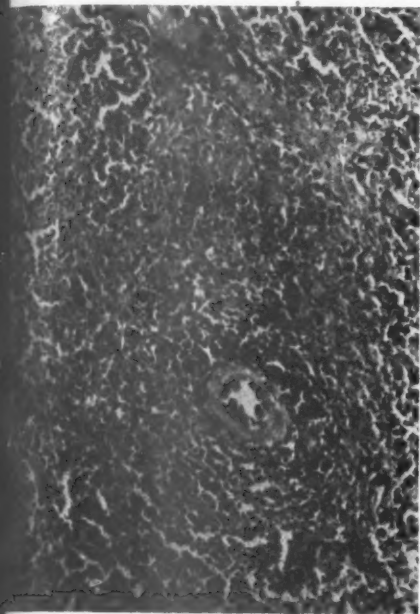
PLATE 136

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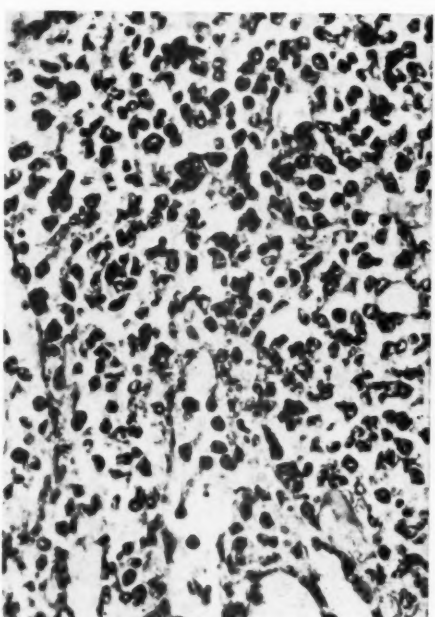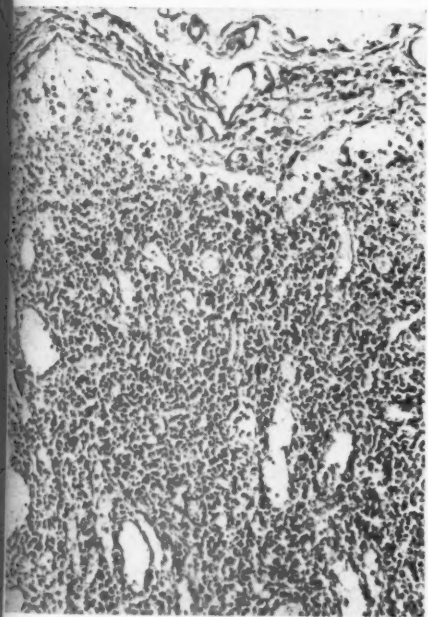
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PLATE 137

FIG. 51. Group II. Lymph node. General view. Absence of secondary follicles. Very few small lymphocytes remain. Numerous plasma cells and larger cells intermediate in appearance between them and the elements of the reticulum. Large mononuclear cells with vacuolated cytoplasm in peripheral sinusoid. Thickening of capsule by granulation tissue. K-30. Nagashima. Male, 23 or 28 years of age (variously stated). Approximately 1000 yds. Died on the 26th day. A.I.P. neg. HM 213. × 120.

FIG. 52. Group II. Lymph node. Enlargement of a portion of Figure 51, including a subcapsular sinusoid and the underlying parenchyma. A.I.P. neg. HM 214. × 430.

FIG. 53. Group II. Lymph node. Tissue near edge of focus of necrosis. Large mononuclear elements with folded nuclei, probably atypical reticulum cells. Occasional plasma cells. Hemorrhage. K-28. Kawaura. Male, 23 years of age. Approximately 1000 yds. Died on the 26th day. A.I.P. neg. HM 152. × 450.

FIG. 54. Group III. Lymph node. Enormous atypical cells resembling Reed-Sternberg cells. K-82. Oku. Female, 15 years of age. Approximately 1200 yds. Died on the 42nd day. A.I.P. neg. HM 301. × 400.

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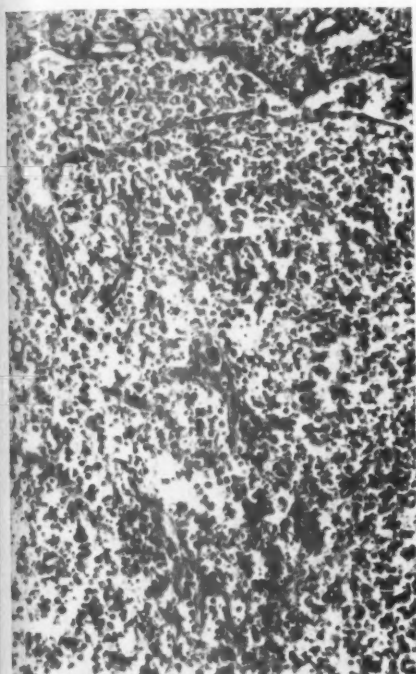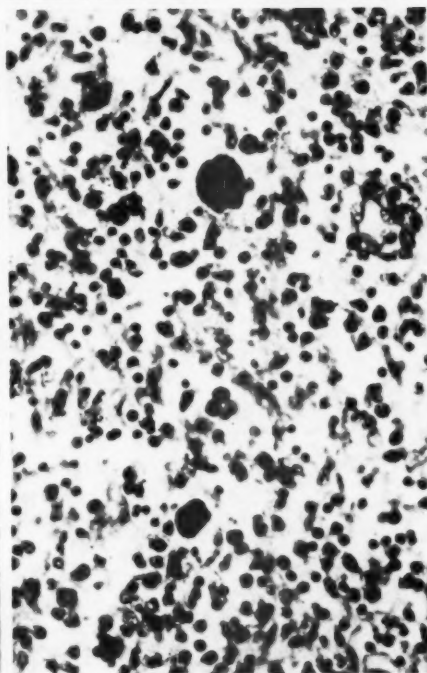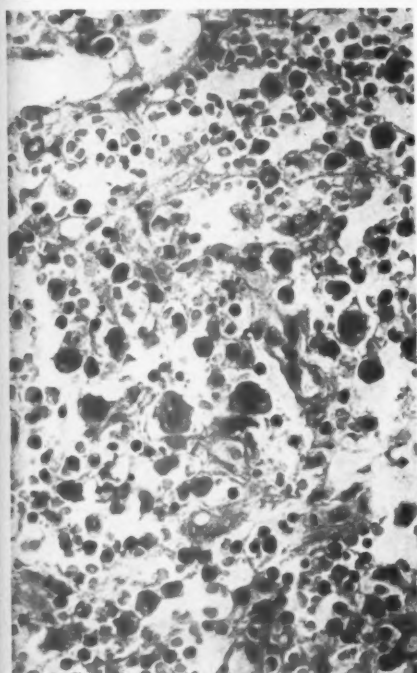
FIG. 55. Group II. Bone marrow, rib. Extreme hypoplasia, "type A" marrow. K-119. Nagado. Female, 26 years of age. Approximately 1300 yds. Died on the 23rd day. A.I.P. neg. HM 340. $\times$ 13.

FIG. 56. Group II. Bone marrow, rib. Hypoplasia, "type A" marrow. Reticulum cells and plasmacytoid elements. Giemsa's stain. K-39. Takeuchi. Male, 29 years of age. Approximately 1000 yds. Died on the 29th day. A.I.P. neg. HM 184. $\times$ 180.
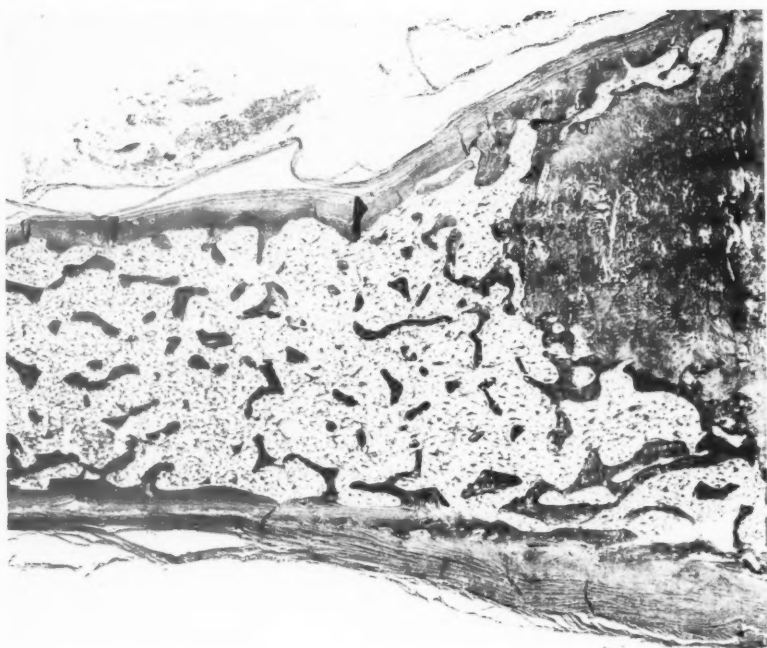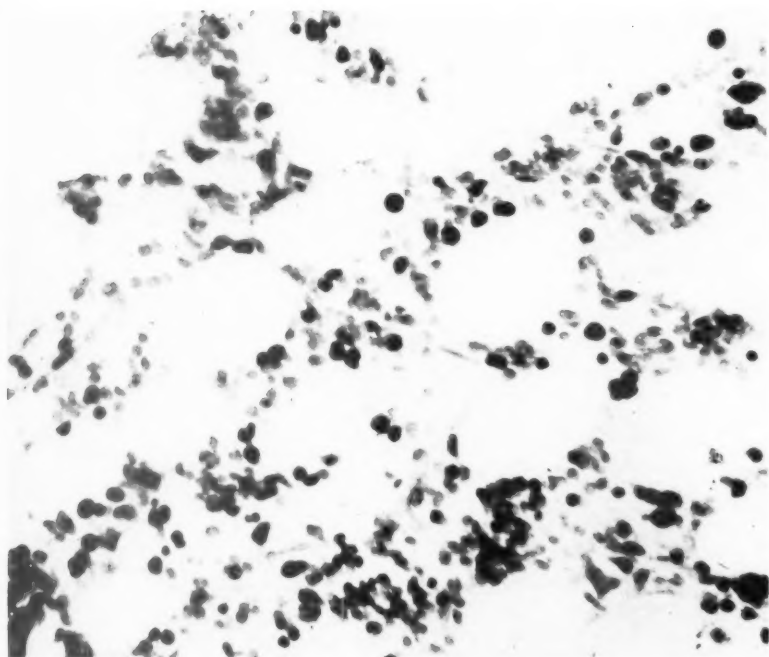
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PLATE 139

FIG. 57. Group III. Rib. Costochondral junction. Hyperplasia of bone marrow, "type C" marrow. K-133. Nagai. Male, 9 years of age. Approximately 500 yds. Died on the 54th day. A.I.P. neg. HM 337. × 13.

FIG. 58. Group I. Bone marrow, sternum. Hypoplasia. Plasma cells arranged adventitially in relation to the sinusoids. Some large cells intermediate in appearance between the plasma cells and elements of the reticulum. This is the earliest histologic specimen of marrow available from a bone in which there is usually active hemopoiesis. Its classification is "type A," marked hypoplasia. K-98. Tamai. Male, 19 years of age. Distance unknown. Died on the tenth day. A.I.P. neg. HM 283. × 400.

FIG. 59. Group I. Bone marrow. Many plasma cells. Focal hyperplasia of reticulum. Some cells intermediate in appearance between plasma cells and elements of the reticulum. The atypical plasmacytoid differentiation is remarkable. No erythropoietic tissue or megakaryocytes are in evidence. This marrow is classified as "type B," marked focal reticulum hyperplasia. K-100. Ueki. Female, 32 years of age. Distance unknown. Died on the twelfth day. A.I.P. neg. HM 284. × 400.
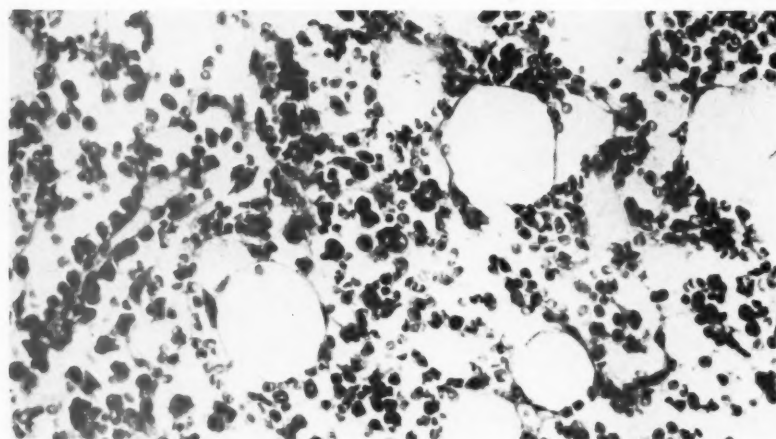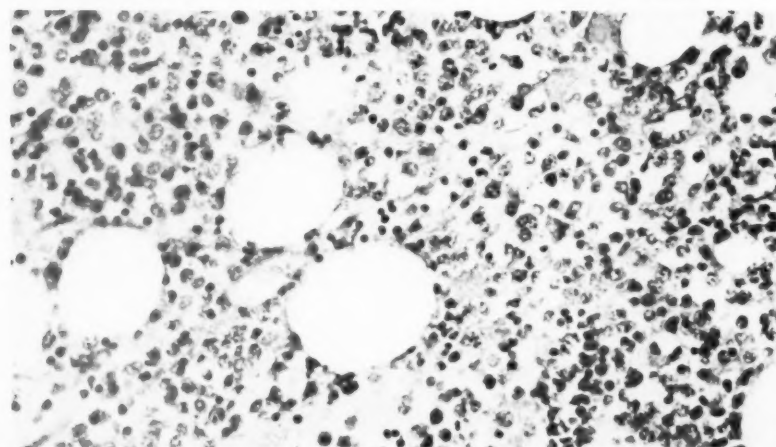
PLATE 140

FIG. 60. Group II. Bone marrow (femoral). General view. Groups of reticulum cells, lymphocytes, and plasmacytoid elements. Islets of erythropoietic tissue persist. "Type A" marrow. K-25. Shintaku. Male, age unknown. Approximately 1000 yds. Died on the 25th day. A.I.P. neg. HM 195. × 230.

FIG. 61. Group II. Bone marrow. Femoral. "Type A" marrow. Reticulum and plasma cells. Erythrophagocytosis. K-28. Kawaura. Male, 23 years of age. Approximately 1000 yds. Died on the 26th day. A.I.P. neg. HM 200. × 1000.

FIG. 62. Group II. Vertebral marrow. Hyperplasia of reticulum cells. "Type B" marrow. K-22. Michihara. Male, 17 or 23 years of age (variously stated). Approximately 1000 yds. Died on the 25th day. A.I.P. neg. HM 147. × 450.

FIG. 63. Group II. Bone marrow. Reticulum cell hyperplasia. "Type B" marrow. Prominent nucleoli in some cells. Some cells are still spindle-shaped and others have become rounded. Some plasma cells are present. Giemsa's stain. K-41. Takano. Male, 23 years of age. Approximately 1000 yds. Died on the 30th day. A.I.P. neg. HM 324. × 1020.

FIG. 64. Group II. Reticulum hyperplasia. Giant cell, possibly immature megakaryocyte. From the same patient as Figure 63. A.I.P. neg. HM 323. × 1020.

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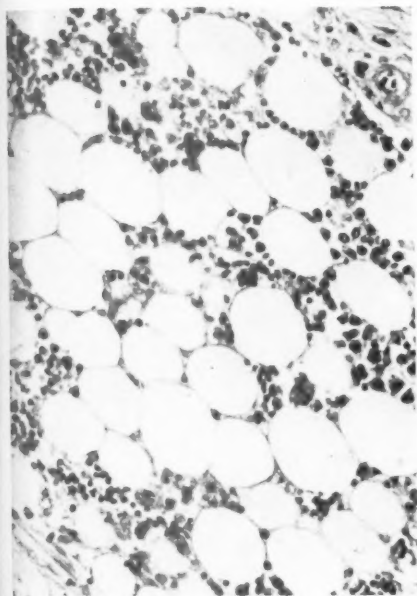
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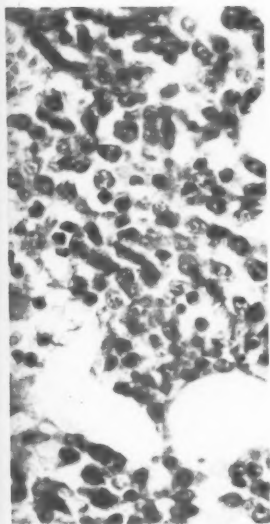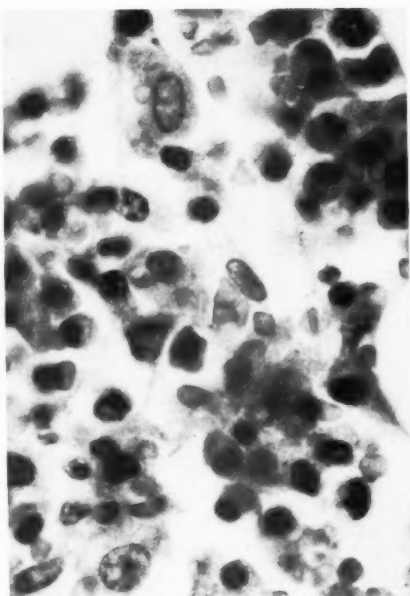
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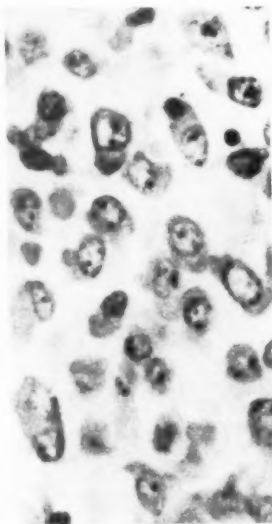
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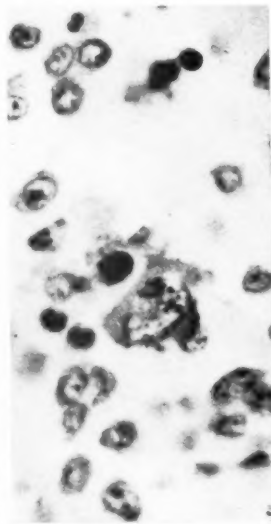
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PLATE 141

FIG. 65. Group II. Bone marrow, sternum. Reticulum cells, young myelocytes, and plasma cells. K-27. Omura. Male, 22 years of age. Approximately 1000 yds. Died on the 26th day. A.I.P. neg. HM 250. × 1000.

FIG. 66. Group II. Bone marrow, vertebral. Streptococci in wall of sinusoid, without local tissue response. Numerous large reticulum cells. Giemsa's stain. K-22. Michihara. Male, 23 or 17 years of age (variously stated). Died on the 25th day. A.I.P. neg. HM 183. × 1100.

FIG. 67. Group II. Bone marrow, rib. Focus of necrosis. Masses of bacteria. Giemsa's stain. K-39. Takeuchi. Male, 29 years of age. Approximately 1000 yds. Died on the 29th day. A.I.P. neg. HM 197. × 230.

FIG. 68. Group II. Bone marrow, rib. Bacilli in large numbers, without cellular reaction. Giemsa's stain. Enlargement of a portion of Figure 67. A.I.P. neg. HM 198. × 1000.

FIG. 69. Group III. Hyperplasia of reticulum. Some cells have more prominent nucleoli than are seen in the typical reticulum cell and may be forms in transition to myelocytes, although the granules in the cytoplasm are not seen. Blast cells are rare. K-118. Inaba. Female, 56 years of age. Approximately 700 yds. Died on the 60th day. A.I.P. neg. HM 316. × 750.

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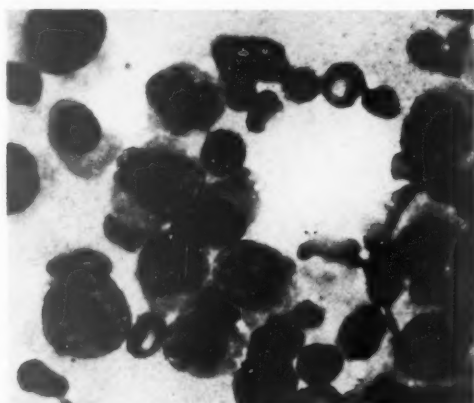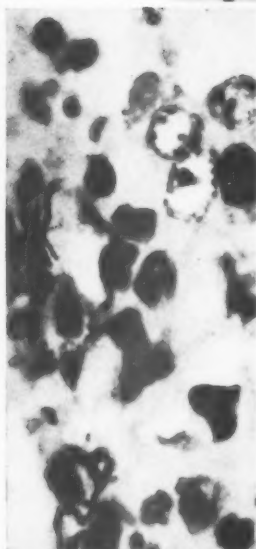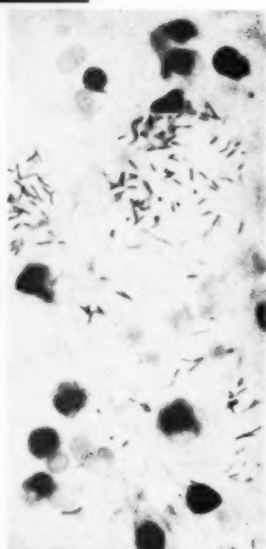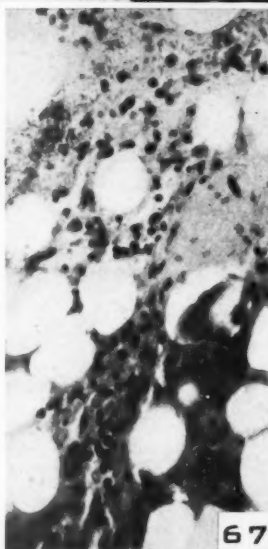
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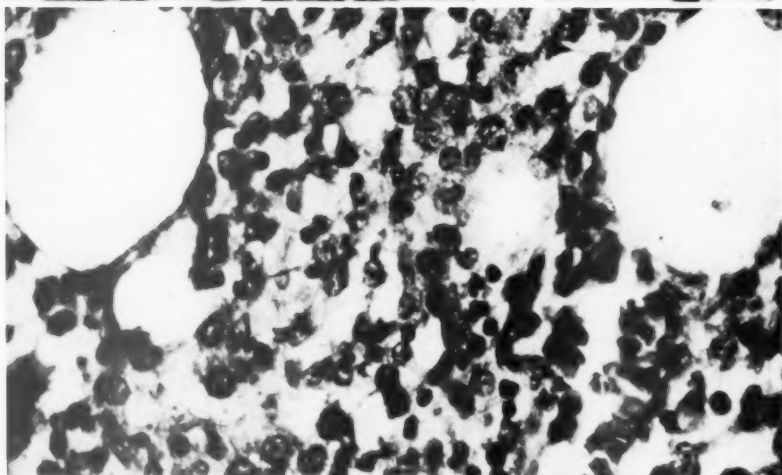
PLATE 142

FIG. 70. Group III. Bone marrow of rib. Focus of regenerating tissue within the gelatinous marrow. "Type C" marrow. The vertebral marrow in this patient was much more cellular. K-50. Kijima. Male, 31 years of age. Approximately 1000 yds. Died on the 100th day. A.I.P. neg. HM 142a. × 115.

FIG. 71. Group III. Bone marrow smear. Myelocytes and a cell with clear cytoplasm resembling that of a lymphocyte. From the same patient as Figure 70. A.I.P. neg. HM 309. × 800.

FIG. 72. Group III. Bone marrow. Extreme hyperplasia, "Type D" marrow. Myelocytes and metamyelocytes predominate. Small islands of erythropoietic tissue. K-14. Yamamoto. Male, 25 years of age. Approximately 1000 yds. Died on the 47th day. A.I.P. neg. HM 261. × 130.

FIG. 73. Group III. Bone marrow, sternal. Fibrin deposited about megakaryocytes. K-110. Watanabe. Male, 56 years of age. Approximately 1000 yds. Died on the 27th day. A.I.P. neg. HM 287. × 500.

FIG. 74. Group III. Bone marrow, rib. "Gelatinous marrow" showing atrophic fat cells, and granular interstitial material containing large quantities of fibrin. Masson's stain. K-50. Kijima. Male, 31 years of age. Approximately 1000 yds. Died on the 100th day. A.I.P. neg. HM 266. × 400.

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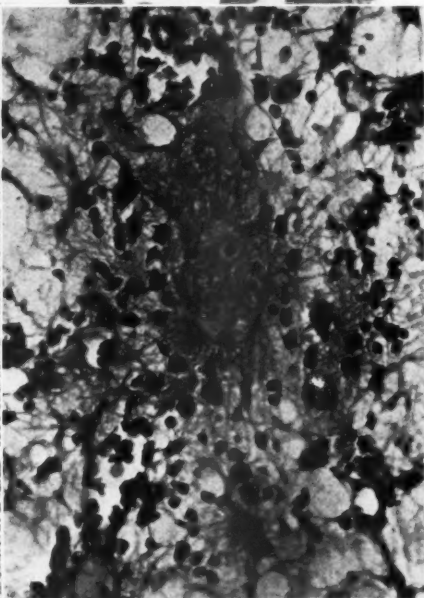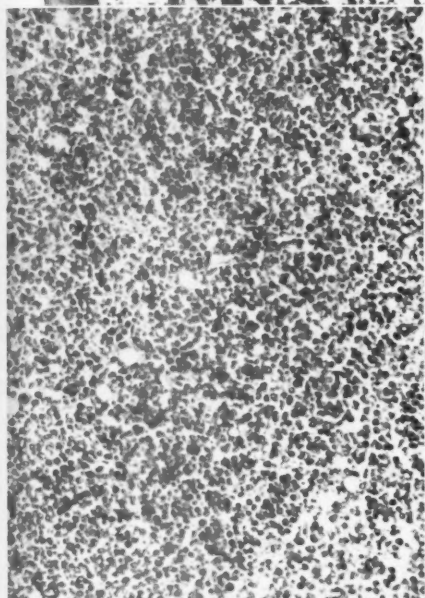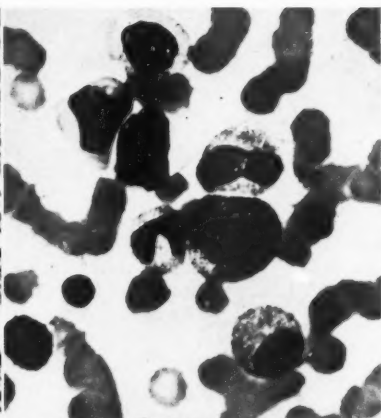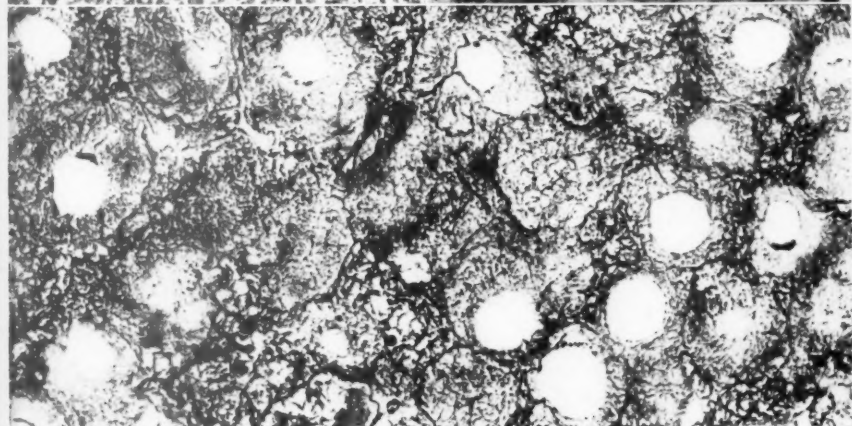
FIG. 75. Group III. Peripheral blood film. Cells of monocytic type. K-224. (Nagasaki.) Matsuo. Male, 19 years of age. Approximately 1000 yds. Died on the 97th day. A.I.P. neg. NM 163. × 1100.

FIG. 76. Group III. Supravital blood film of November 16, 1945, showing phagocytosis of carbon by the atypical mononuclear cells. From the same patient as Figure 75. A.I.P. neg NM 158. Copy of Japanese photograph.

FIG. 77. Group III. Bone marrow (probably from long bone). Large mononuclear cells forming thick septa among the fat cells. From the same patient as Figure 75. A.I.P. neg. NM 164. × 400.

FIG. 78. Group III. Striated muscle. Atypical large mononuclear cells infiltrating among the fibers. Phosphotungstic acid-hematoxylin stain. From the same patient as Figure 75. × 500.

FIG. 79. Group III. Liver. Pericentral infiltration of large mononuclear elements. Atrophy of central ends of hepatic cell cords. From the same patient as Figure 75. A.I.P. neg. NM 162. × 120.

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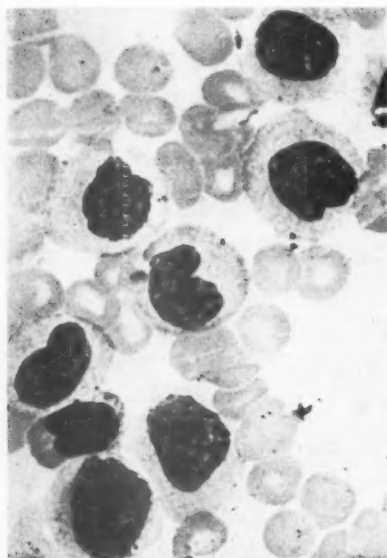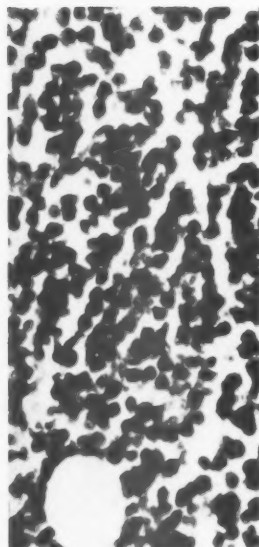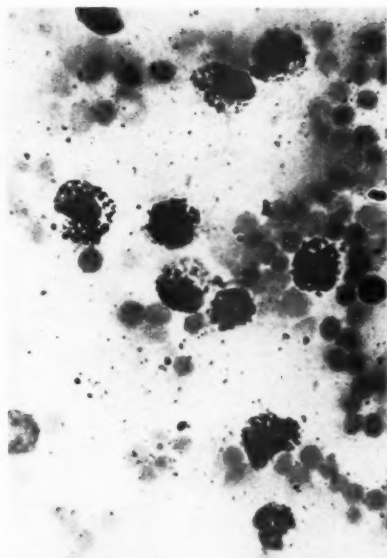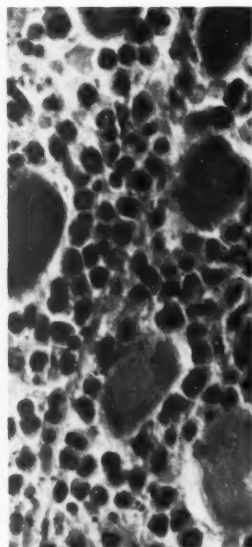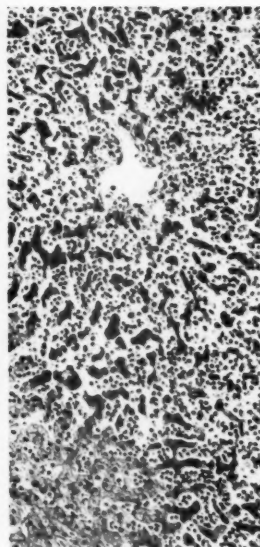
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Pathology of Atomic Bomb Casualties

PLATE 144

FIG. 80. Group I. Ileum. Epithelial cell with enormous nucleus at base of crypt. Abundant plasmacytoid cells in the lamina propria. K-5. Yano. Male, 39 years of age. Approximately 1000 yds. Died on the sixth day. A.I.P. neg. HM 127. × 810.

FIG. 81. Group I. Intestine. Base of crypt. Slightly below and to the left of the center there is an epithelial cell in tripolar mitosis. K-2. Onishi. Male, 24 years of age. Approximately 800 yds. Died on the fourth day. A.I.P. neg. HM 125. × 650.
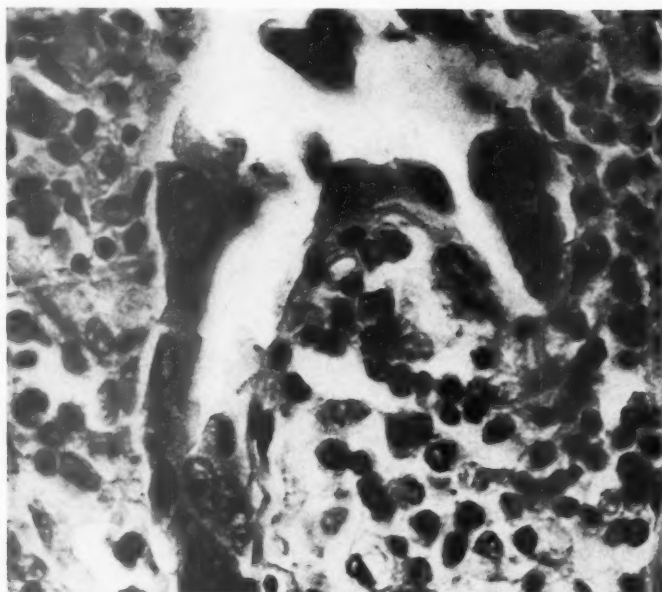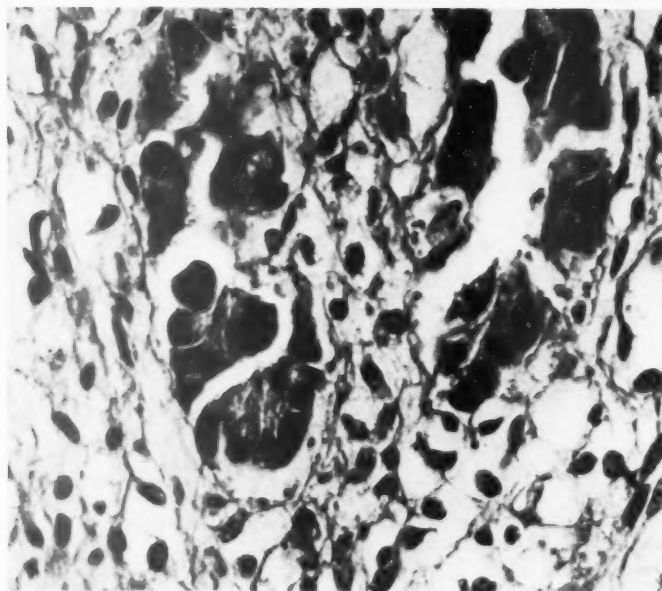
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FIG. 82. Group I. Intestine. Atypical epithelial cells at base of crypt. K-175. (Nagasaki.) Yamada. Female, 18 years of age. Distance unknown. Died on the eleventh day. A.I.P. neg. NM 159. × 600.

FIG. 83. Group I. Intestine. Superficial ulcers containing masses of bacteria. Edema of submucosa. No leukocytic infiltration. From the same patient as Figure 82. × 25.
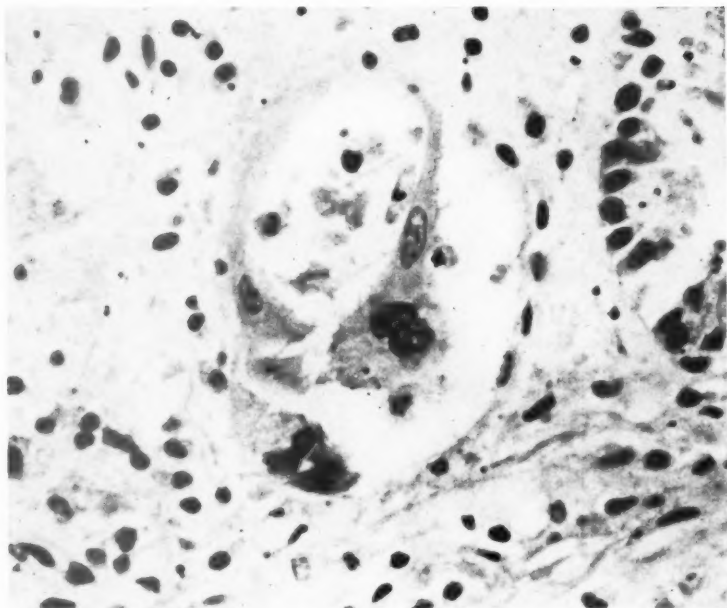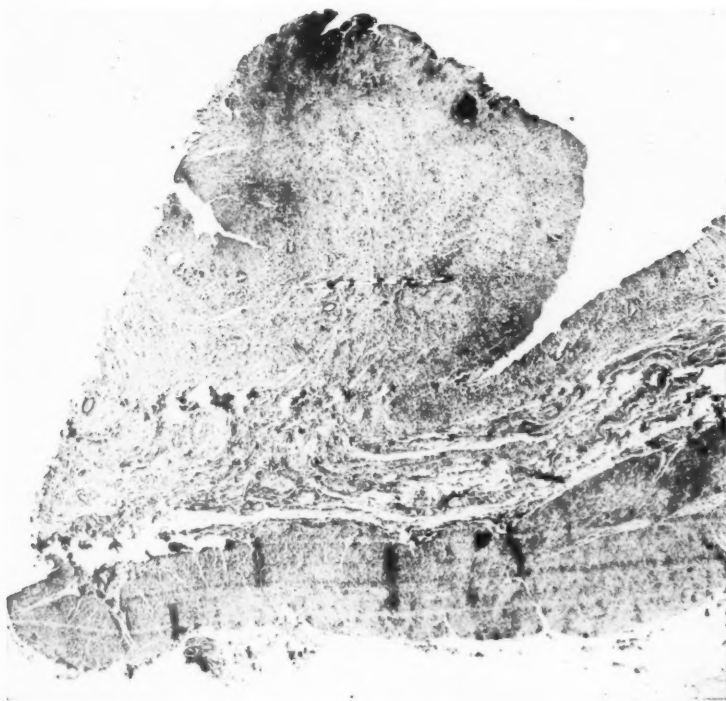
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FIG. 84. Group II. Stomach. Edema, hemorrhage, necrosis, and superficial ulcers of mucosa of stomach. The process is diffuse, except for a narrow band of well preserved mucous membrane along the lesser curvature and in a few places elsewhere. K-21. Iseoka. Male, 45 years of age. Approximately 1000 yds. Died on the 24th day. A.I.P. neg. HS 301.

FIG. 85. Group II. Ileocecal valve. Necrosis and hemorrhage of ileocecal valve and ascending colon. K-37. Takeda. Male, 25 years of age. Approximately 1000 yds. Died on the 29th day. A.I.P. neg. HS 320.
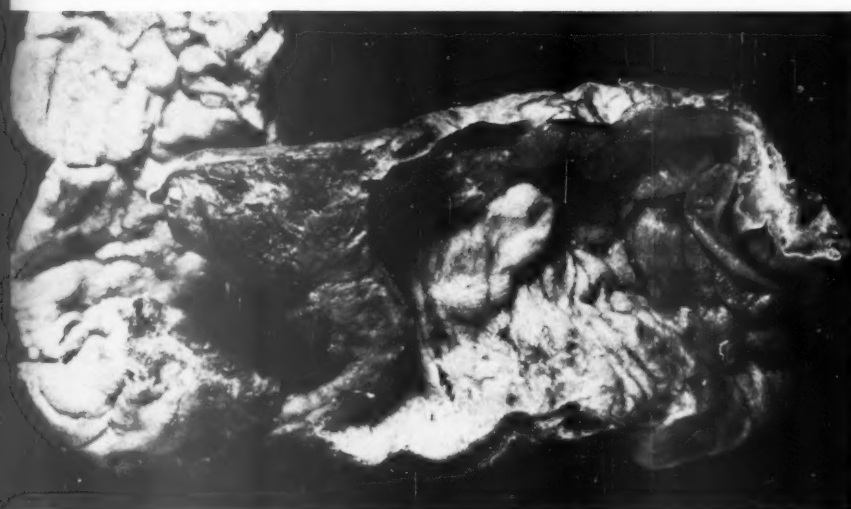
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FIG. 86. Group II. Intestine. Focal hemorrhages of ileum. Hemorrhages and ulcers of ascending colon and ileocecal valve. K-35, Takahashi. Male, 31 years of age. Approximately 1000 yds. Died on the 28th day. A.I.P. neg. HS 317.

FIG. 87. Intestine. Necrosis and hemorrhage of mucous membrane. Ileocecal valve involved. K-27. Omura. Male, 22 years of age. Approximately 1000 yds. Died on the 26th day. A.I.P. neg. HS 306.

FIG. 88. Group II. Large intestine. Ulceration of mucous membrane. Fibrinous exudate adheres in large, irregular shreds. Diffuse hemorrhage of submucosa. K-38. Kamihara. Male, 22 years of age. Approximately 1000 yds. Died on the 29th day. A.I.P. neg. HS 321.

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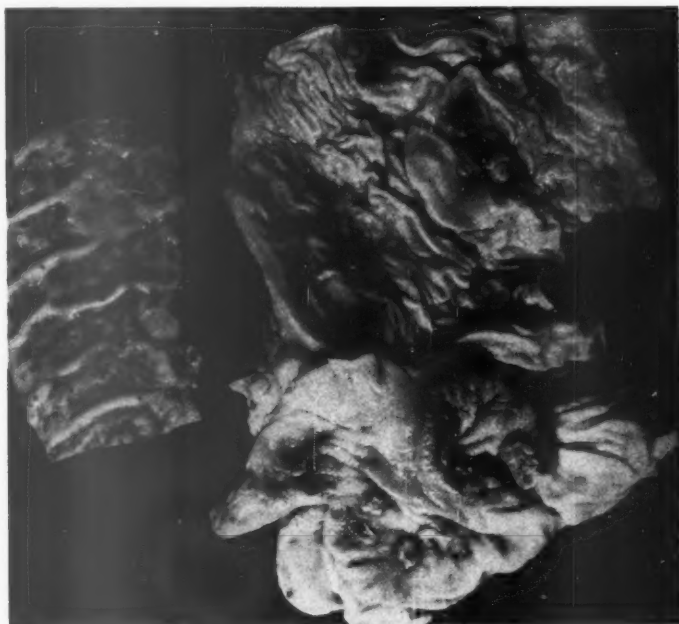
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PLATE 148

FIG. 89. Group II. Stomach. Focal necrosis. Bacterial masses at surface. Plasma cell infiltration of wall. From the same patient as Figure 84. A.I.P. neg. HM 146. × 81.

FIG. 90. Group II. Margin of ulcer. The exudate consists of small and large mononuclear cells and plasma cells, without polymorphonuclear leukocytes. K-43. Horinouchi. Male, 33 years of age. Approximately 1000 yds. Died on the 33rd day. A.I.P. neg. HM 235. × 130.

FIG. 91. Group II. Colon. Polypoid mass of necrotic tissue. Edema of mucosa and submucosa. No leukocytic infiltration.

FIG. 92. Group III. Colon. Amebae in edematous areolar tissue of the submucosa. K-131. Takatani. Female, 39 years of age. Approximately 1500 yds. Died on the 46th day. A.I.P. neg. HM 339. × 350.

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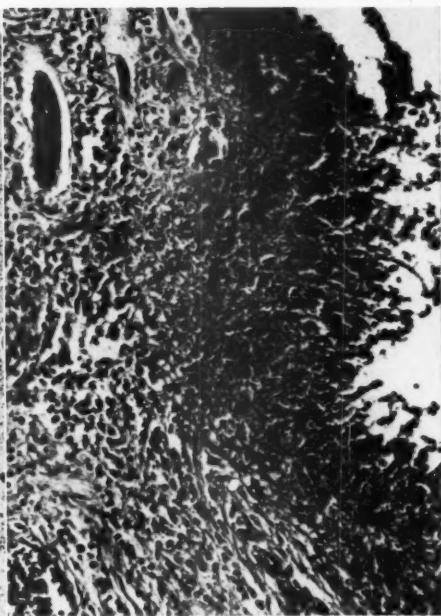
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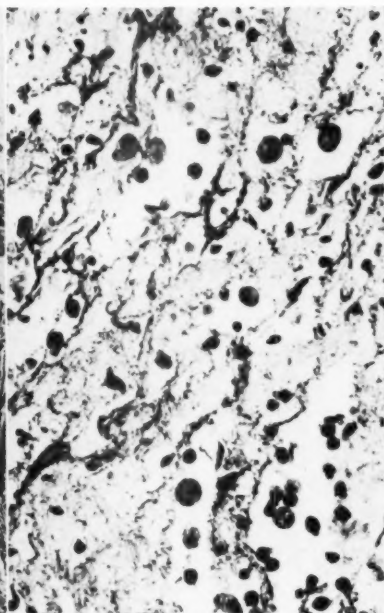
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FIG. 93. Group I. Liver. Central congestion. Edema of connective tissue about the central vein. Large nuclei in the cells of the cords nearest the central venules. K-2. Onishi. Male, 24 years of age. Approximately 800 yds. Died on the fourth day. × 250.

FIG. 94. Group II. Liver. Focus of necrosis. The cellular exudate is scanty and consists almost entirely of plasma cells, many of which are represented only by granular pyknotic débris. K-89. Kuroki. Male, 23 years of age. Approximately 1000 yds. Died on the 21st day. A.I.P. neg. HM 273. × 200.

FIG. 95. Group III. Liver. Central necrosis; thickening of wall of central venule. Exudate of small and large mononuclear cells. K-14. Yamamoto. Male. 25 years of age. Approximately 1000 yds. Died on the 47th day. A.I.P. neg. HM 212. × 115.
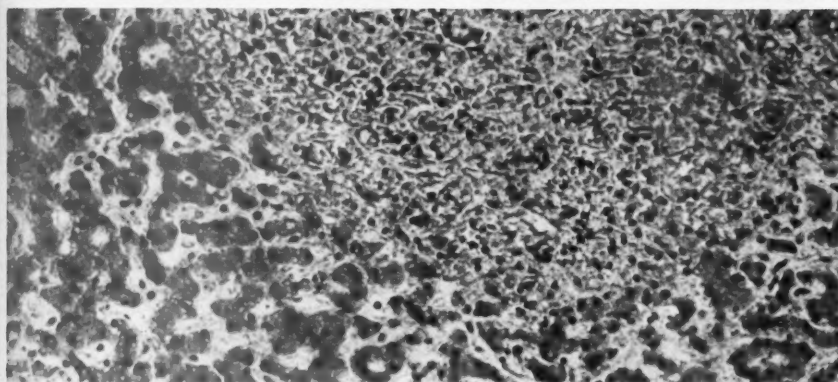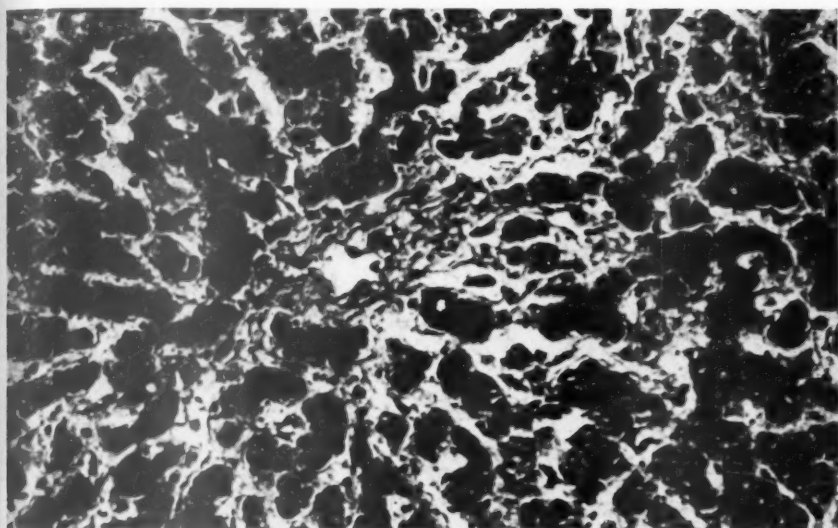
Liebow, Warren, and DeCoursey   Pathology of Atomic Bomb Casualties

FIG. 96. Group I. Kidney. Sinusoids at corticomedullary junction containing large mononuclear cells, one in mitosis. K-9. Sakamoto. Male, 25 years of age. Approximately 1500 yds. Died on the eighth day. A.I.P. neg. HM 137. $\times$ 400.

FIG. 97. Group II. Kidney. Petechiae in parenchyma of kidney, hemorrhage in mucous membrane of pelvis. There was no evidence of glomerulonephritis histologically. K-30. Nagashima. Male, 23 or 28 years of age (variously stated). Approximately 1000 yds. Died on the 26th day. A.I.P. neg. HS 312.
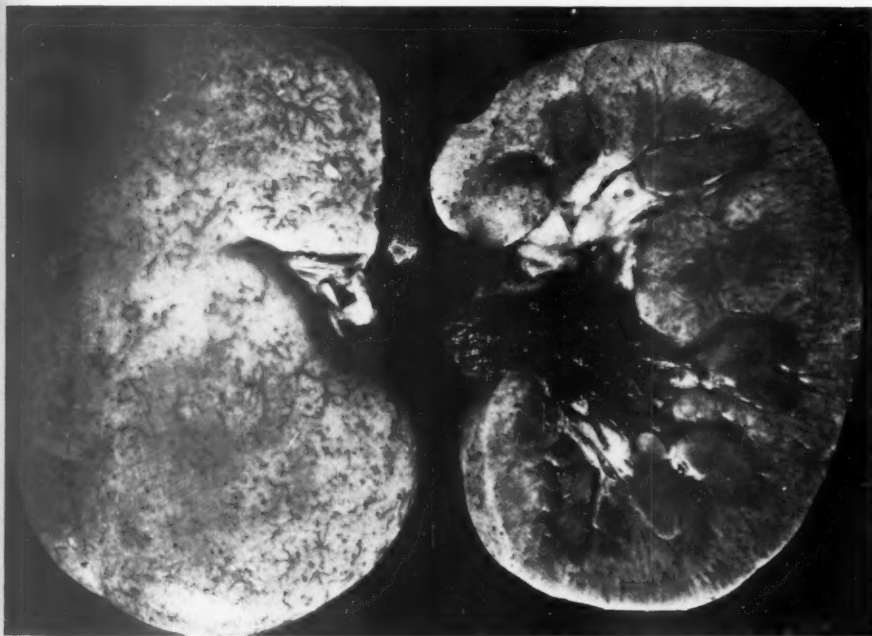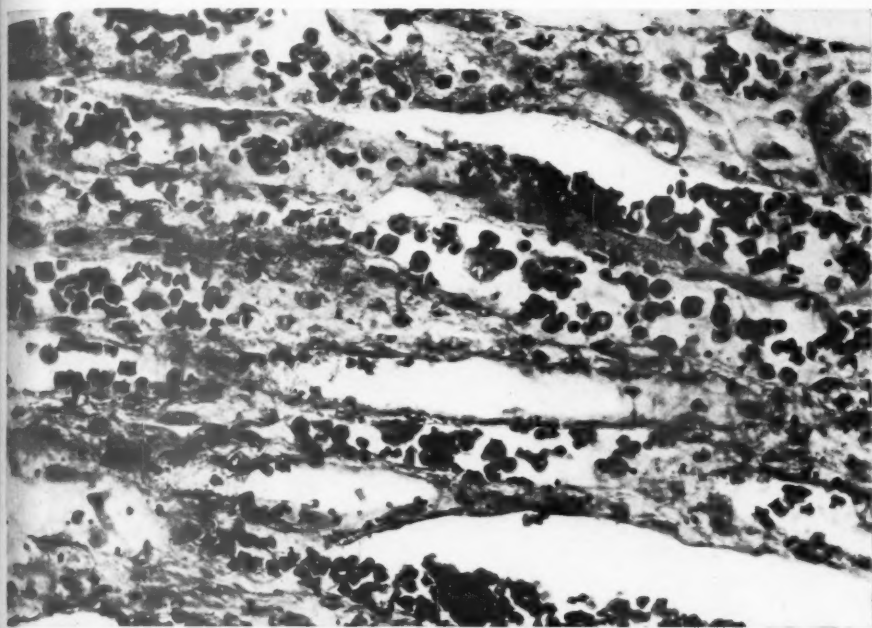
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Pathology of Atomic Bomb Casualties

PLATE 151

FIG. 98. Group I. Testis. Sloughing of spermatogonia from basement membrane whereon rest Sertoli cells in increased numbers. Relatively few spermatids and mature spermatozoa in lumina of tubules. Mitotic figures are relatively rare. K-2. Onishi, 24 years of age. Approximately 800 yds. Died on the fourth day. A.I.P. neg. HM 122. × 160.

FIG. 99. Group II. Testes. Atrophy. K-30. Nagashima, 23 or 28 years of age (variously stated). Died on the 26th day. A.I.P. neg. HS 313.

FIG. 100. Group II. Testis. Atrophy. Necrotic remnants of germinal epithelium and its derivatives in lumina of tubules. Edema of interstitial tissues. Hyaline subendothelial changes of arterioles. K-28. Kawaura, 23 years of age. Approximately 1000 yds. Died on the 26th day. A.I.P. neg. HM 150. × 125.
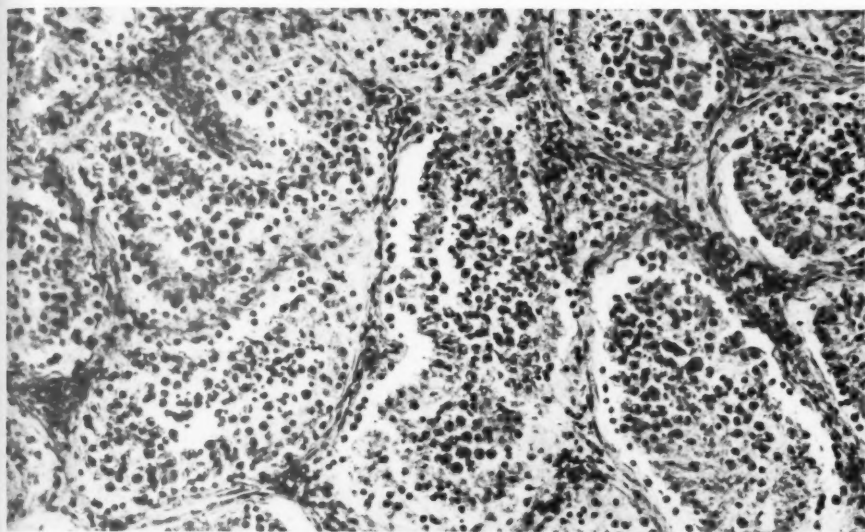
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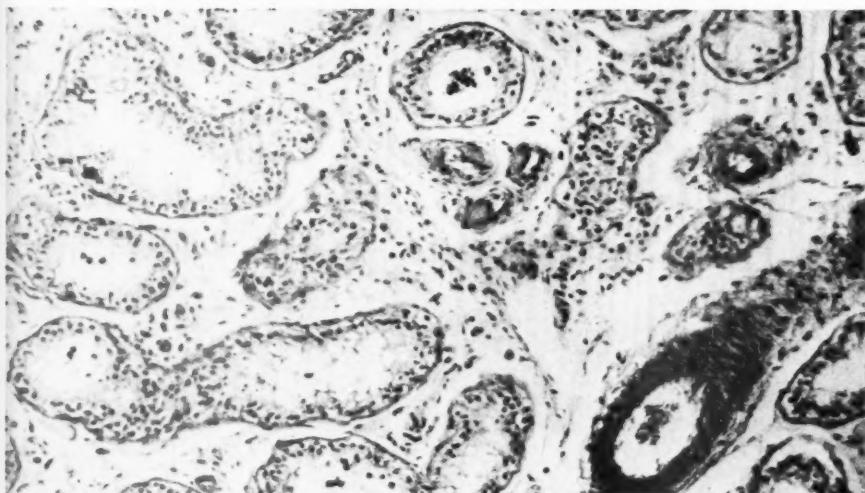
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FIG. 101. Group II. Testis. Atrophy of germinal epithelium and its derivatives. Hyperplasia of interstitial tissue. K-39. Takeuchi, 29 years of age. Approximately 1000 yds. Died on the 29th day. A.I.P. neg. HM 228. × 115.

FIG. 102. Group II. Testis. Atrophy of tubules. Near the basement membrane, among the Sertoli cells, is an ovoid cell with a hyperchromatic nucleus, considered to be a persistent element of the germinal epithelium. K-46. Kurihara, 22 years of age. Approximately 800 yds. Died on the 33rd day. A.I.P. neg. HM 241. × 450.

FIG. 103. Group II. Testis. Atrophy. Tubule containing "giant cell" apparently produced by fusion and compaction of the cytoplasm of spermatids, some of which are still isolated. K-40. Motoyama, 29 years of age. Approximately 1000 yds. Died on the 30th day. A.I.P. neg HM 231. × 450.

FIG. 104. Group II. Testis. Atrophy. "Giant cell" in lumen of tubule, which is lined by Sertoli cells. The "giant" cell probably has resulted from compaction and fusion of spermatids. K-91. Kato, 24 years of age. Approximately 1000 yds. Died on the 30th day. A.I.P. neg. HM 274. × 400.
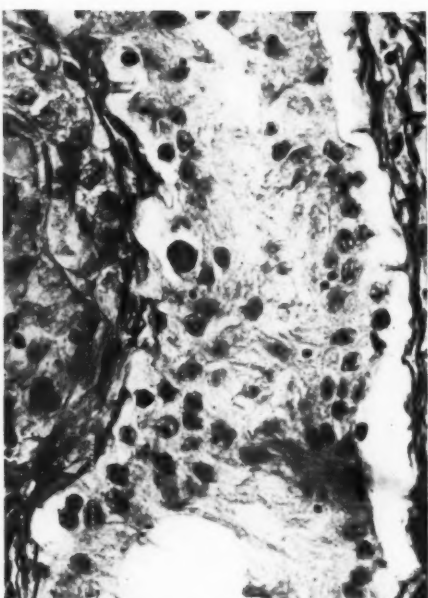
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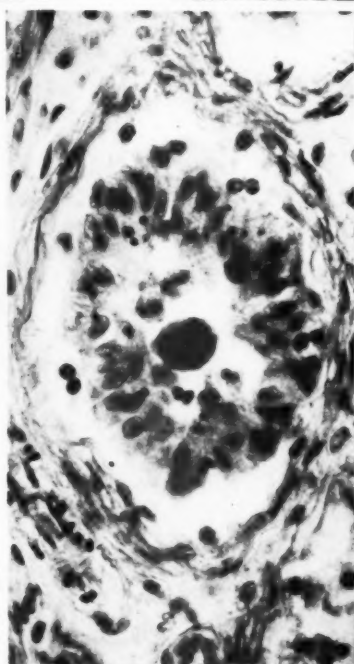
PLATE 153

Fig. 105. Group III. Testis. Cessation of spermatogenesis. The tubules, whose basement membranes have not become thickened, are lined exclusively by Sertoli cells. The interstitial tissue is not hyperplastic. K-50. Kijima, 31 years of age. Approximately 1000 yds. Died on the 100th day. A.I.P. neg. HM 143. × 115.

Fig. 106. Group III. Immature testis. Thickening of basement membranes of tubules. The epithelium appears slightly shrunken but otherwise well preserved. K-128. Fukuhara, 8 years of age. Between 500 and 1000 yds. Died on the 40th day. A.I.P. neg. HM 338. × 100.

Fig. 107. Group III. Prostate. Atrophy. Grossly, this prostate was approximately one-half the usual size. From the same patient as Figure 105. A.I.P. neg. HM 144. × 95.

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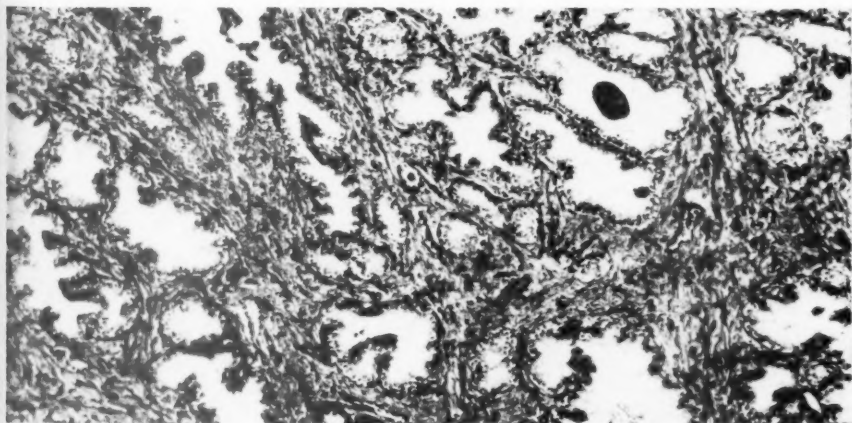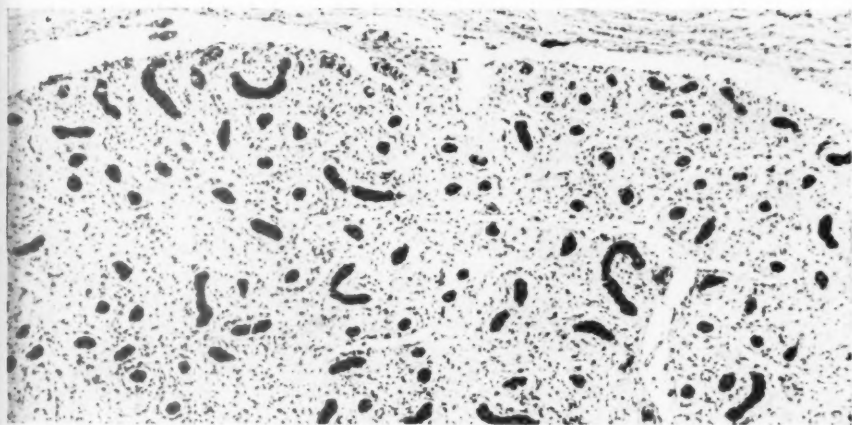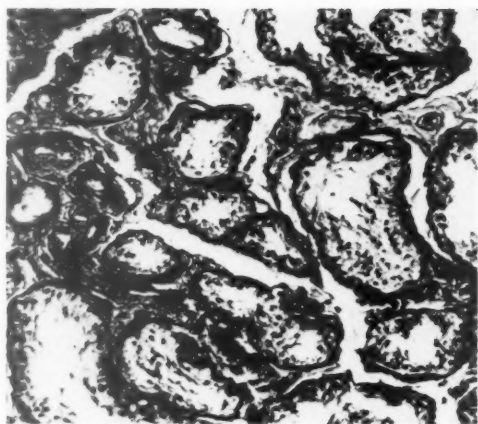Liebow, Warren, and DeCoursey

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Pathology of Atomic Bomb Casualties

FIG. 113. Group II. Sub-pial hemorrhage of frontal lobe. Massive hemorrhage in medullary cistern of sub-arachnoid space. Petechiae of sub-ependyma of lateral ventricle. K-42. Moriseko. Male, 27 years of age. Approximately 1000 yds. Died on the 31st day. A.I.P. neg. HS 324.

FIG. 114. Group II. Brain. Focus of necrosis in medulla, surrounded by hemorrhage. A small bacterial mass which appears black in the photomicrograph lies near the center of the lesion. K-60. Shigeta. Female, 44 years of age. Approximately 800 yds. Died on the 36th day. A.I.P. neg. HM 333. × 100.

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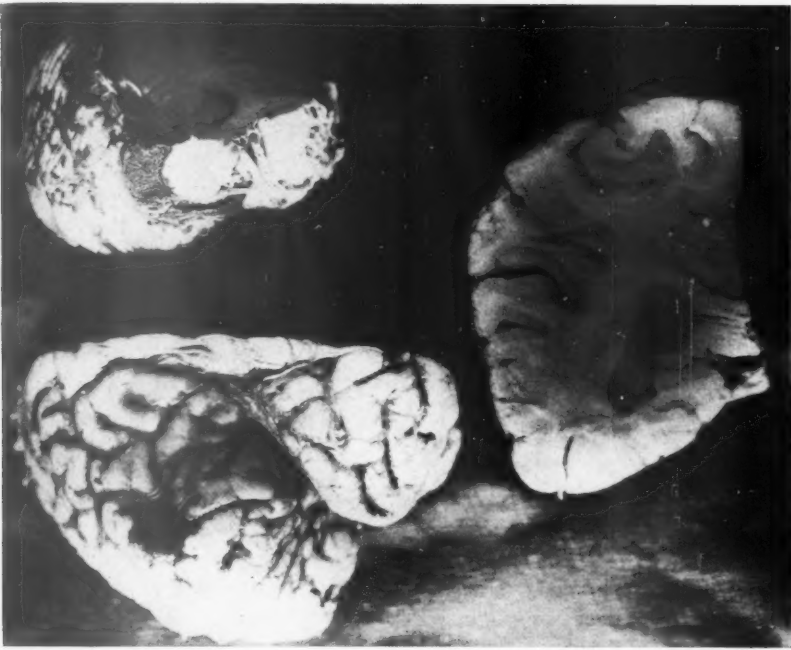
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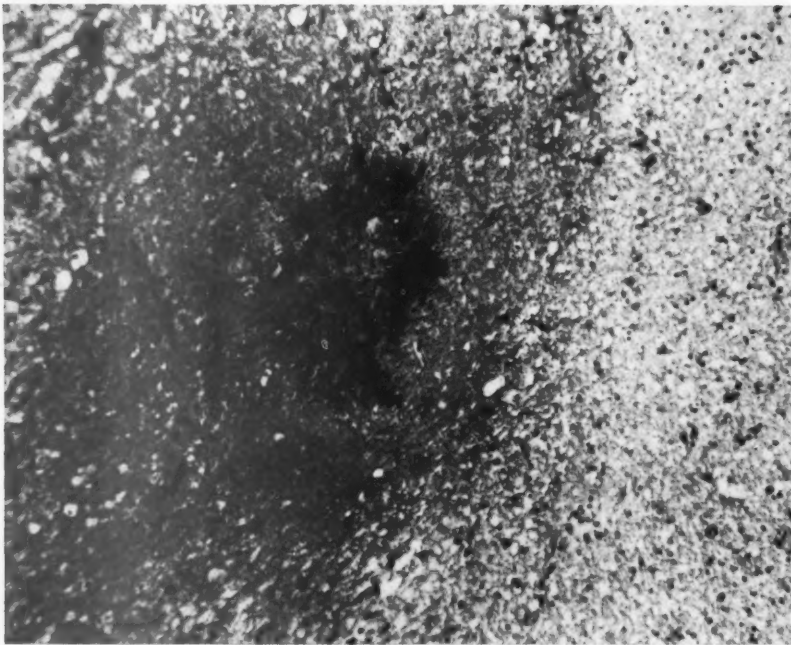
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PLATE 157

FIG. 115. Group III. Adrenal. Focal atrophy with decrease in thickness of cortex. Focal fatty changes in cortex. K-96. Sakoda. Male, 33 years of age. Approximately 1000 yds. Died on the 97th day. × 130.

FIG. 116. Group II. Atrophy of zona glomerulosa. Loss of lipid. Edema of subcapsular connective tissue. Masson's stain. K-2. Onishi. Male, 24 years of age. Approximately 800 yds. Died on the fourth day. A.I.P. neg. HM 124. × 60.

FIG. 117. Group II. Adrenal. Focus of necrosis in fascicular stratum of cortex. Infiltration of large fat-filled phagocytes. K-45. Akagi. Male, 28 years of age. Approximately 1000 yds. Died on the 33rd day. A.I.P. neg. HM 236. × 265.

FIG. 118. Group II. Adrenal. Fatty changes of scattered cells and groups of cells in the cortex. Several epithelial cells in the fascicular stratum are in mitosis. From the same patient as Figure 117. A.I.P. neg. HM 237. × 210.
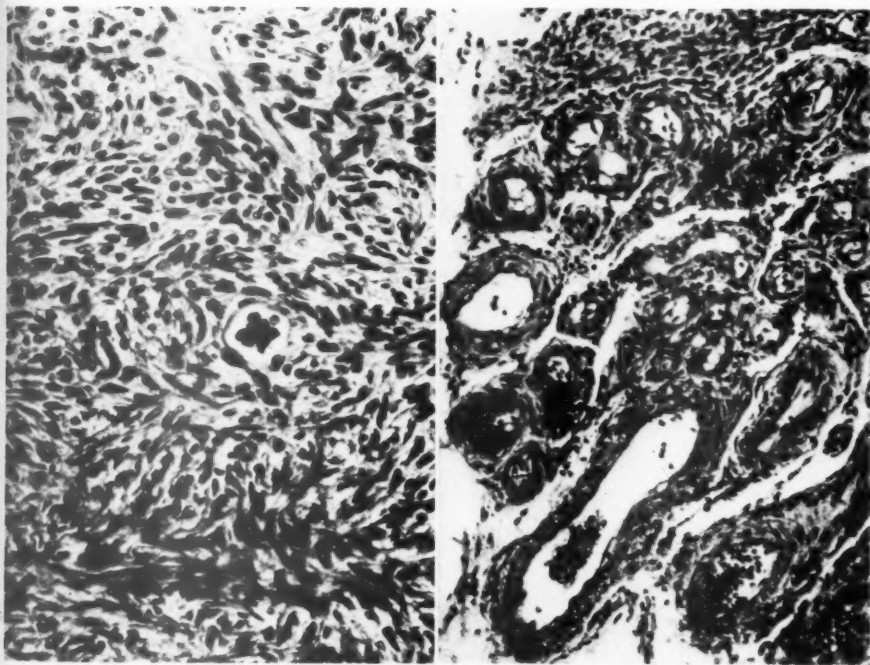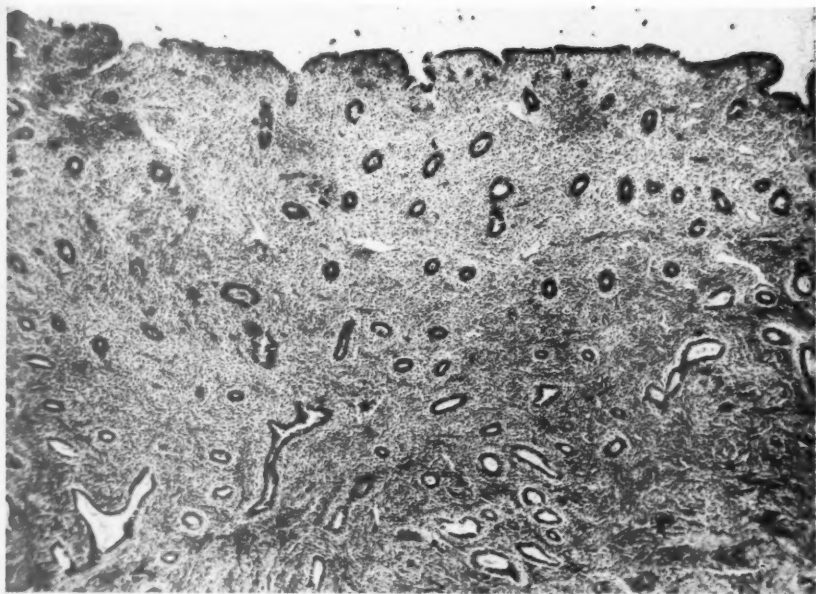
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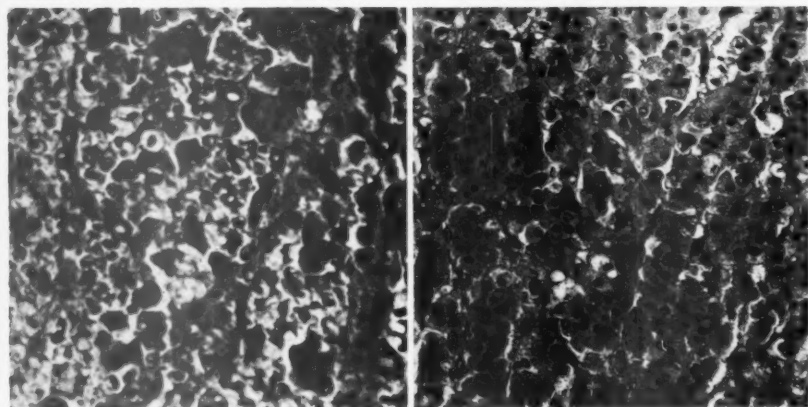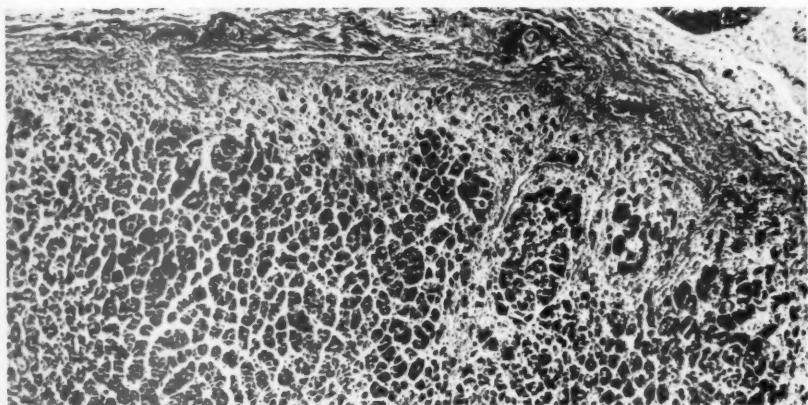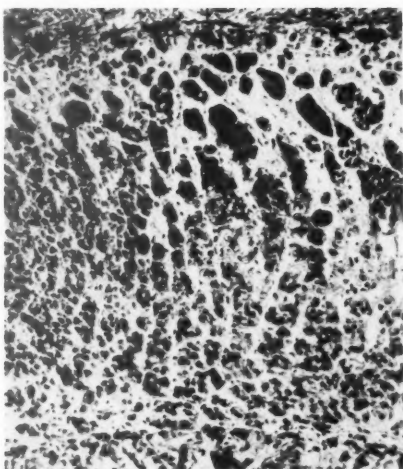
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PLATE 158

FIG. 119. Group I. Wall of pharynx. Swelling, vacuolation, fragmentation, and desquamation of the squamous epithelium. Edema of connective tissue. Atrophy of lymphoid tissue. Tremendous lymphectasia. Occasional plasma cells, mast cells, and large mononuclear elements scattered throughout the areolar tissue. K-98. Tamai. Male, 19 years of age. Distance unknown. Died on the tenth day. × 100.

FIG. 120. Group I. Pharynx. Epithelial swelling and fragmentation. Edema of deeper layers of wall. Enlargement of a portion of Figure 119. A.I.P. neg. HM 332. × 450.

FIG. 121. Group I. Swelling and vacuolation of some squamous epithelial cells. Atrophy of others. Swelling and loss of staining qualities of nuclei. Micronuclei (?) in one cell near surface. Parakeratosis at surface. Dilatation of lymphatics and edema of tongue. From the same patient as Figure 119. A.I.P. neg. HM 330a. × 130.

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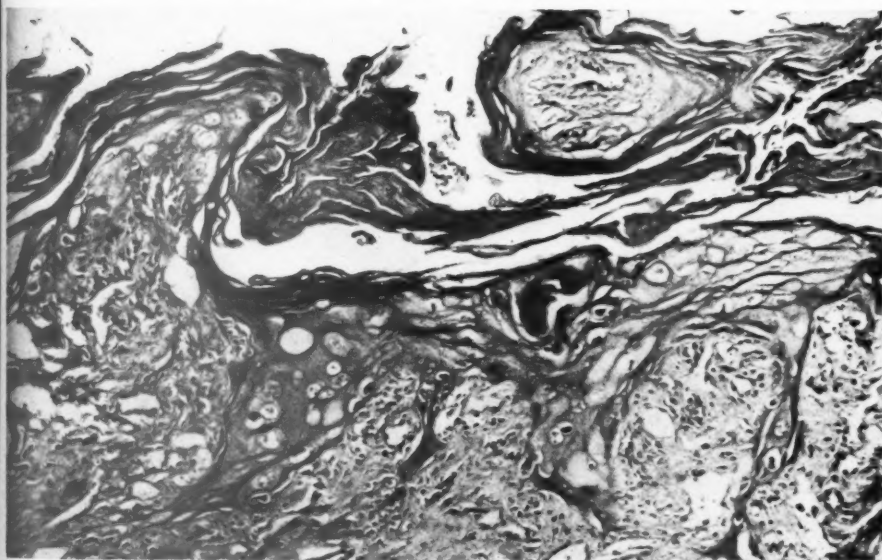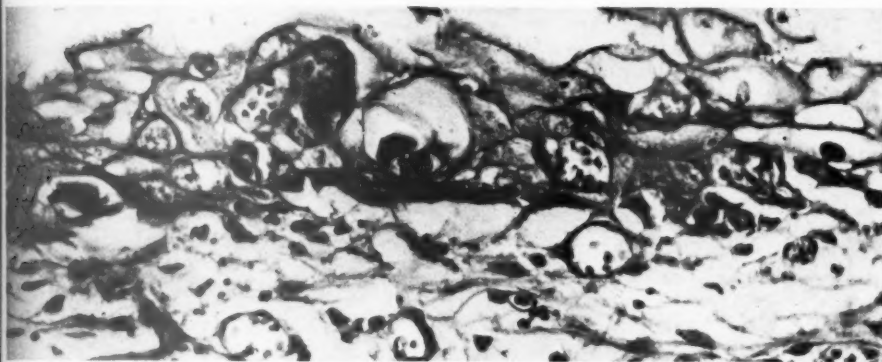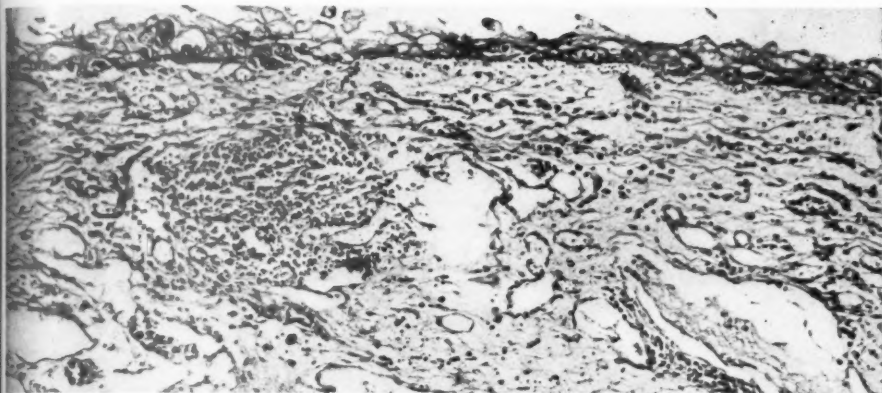
PLATE 159

FIG. 122. Group II. Neck organs. Necrosis of lining membrane of epiglottis, larynx, and trachea. Necrosis and enlargement of left tonsil. Foci of necrosis in the right tonsil, which is somewhat smaller. K-21. Iseoka. Male, 45 years of age. Approximately 1000 yds. Died on the 24th day. A.I.P. neg. HS 300.

FIG. 123. Group II. Neck organs. Necrosis and hemorrhage of faucial and lingual tonsils, pyriform sinus, and epiglottis. K-44. Araki. Male, 22 years of age. Approximately 1000 yds. Died on the 33rd day. A.I.P. neg. HS 328.

FIG. 124. Group II. Tongue. Necrosis and hemorrhage. K-29. Murakami. Male, 22 or 24 years of age (variously stated). Approximately 1000 yds. Died on the 26th day. A.I.P. neg. HS 310.

FIG. 125. Group II. Tonsil. Necrosis. Absence of polymorphonuclear leukocytic barrier between the necrotic and non-necrotic tissue. K-47. Naka. Female, 35 years of age. Approximately 800 yds. Died on the 18th day. A.I.P. neg. HM 264. × 130.

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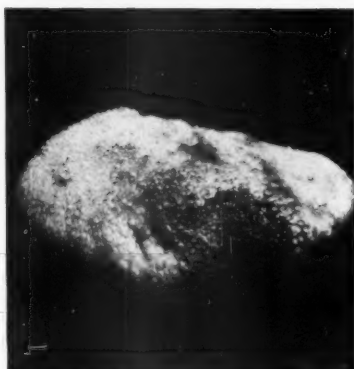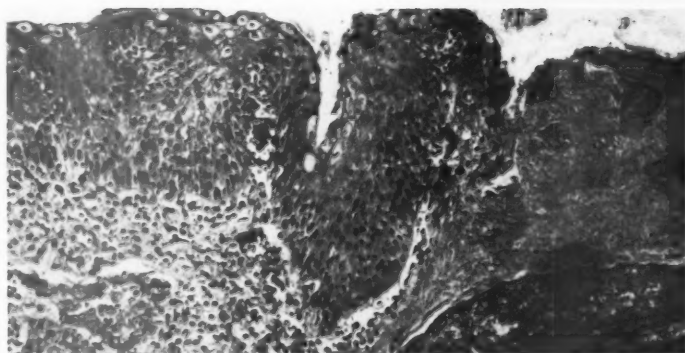
FIG. 126. Group II. Epiglottis. Necrosis and infiltration of small mononuclear and plasma cells. Occasional polymorphonuclear leukocytes. Cartilage intact. K-56. Kawamura. Female, 51 years of age. Approximately 1100 yds. Died on the 36th day. A.I.P. neg. HM 270. × 115.

FIG. 127. Group II. Pharynx. Thrush. Mycelium (probably of monilia). From the same patient as Figure 126. A.I.P. neg. HM 271. × 130.

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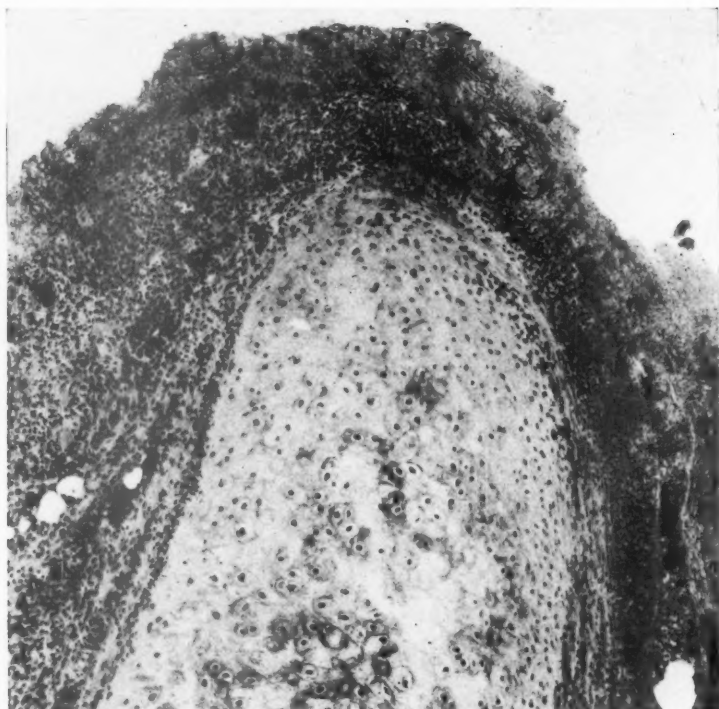
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Pathology of Atomic Bomb Casualties

PLATE 161

FIG. 128. Group II. Patient with petechiae of skin. Epilation appeared on August 18, gingival hemorrhages and petechiae of skin began on August 29. Step-like rise of temperature began on August 31. Necrotizing tonsillitis was noted on September 1, delirium on September 2, and death occurred on September 3, 1945. Laboratory data on day of death: red blood cells. 2.03 millions; hemoglobin, 40 per cent; white blood cells, 1900; platelets, 10,400; bleeding time, 46 minutes. Hashimoto. Male, 21 years of age. Exact distance unknown. No record of autopsy. Photograph made by Japanese medical officers of Tokyo 1st Military Hospital 2 hours before the patient expired. A.I.P. neg. HP 135.

FIG. 129. Group II. Skin. Low-power view of margin of ulcer, and adjacent tissues. K-47. Naka. Female, 35 years of age. Approximately 800 yds. Died on the 18th day. A.I.P. neg. HM 344. × 30.

FIG. 130. Group II. Skin. Ulcer. Bacterial masses in necrotic material. Plasma cells, large mononuclear cells and extravasated erythrocytes at base. K-29. Murakami. Male, 24 or 22 years of age (variously stated). Approximately 1000 yds. Died on the 26th day. A.I.P. neg. HM 296. × 115.

FIG. 131. Group II. Skin. Margin of ulcer. Necrosis of outer layers of epithelium. Pigment remains in basal cells. Congestion of blood vessels and hemorrhages. Bacteria in necrotic material at surface of ulcer. No leukocytic infiltration. Enlargement of a portion of Figure 129. A.I.P. neg. HM 299. × 115.

FIG. 132. Group II. Skin, at distance from ulcer. Vacuolation of epithelial cells and shrinkage of nuclei. Irregularity of distribution of pigment and absence of mitotic figures in basal layer. Small mononuclear cells about dilated blood vessels in corium. Enlargement of a portion of Figure 129. A.I.P. neg. HM 345. × 150.
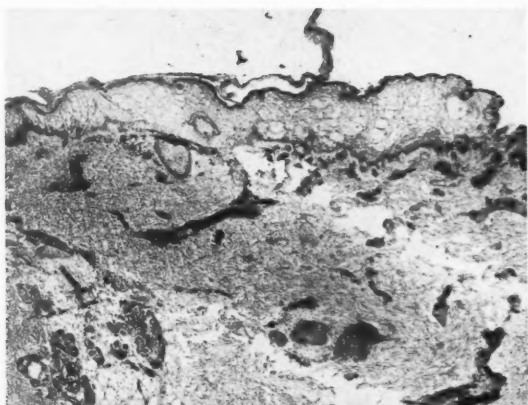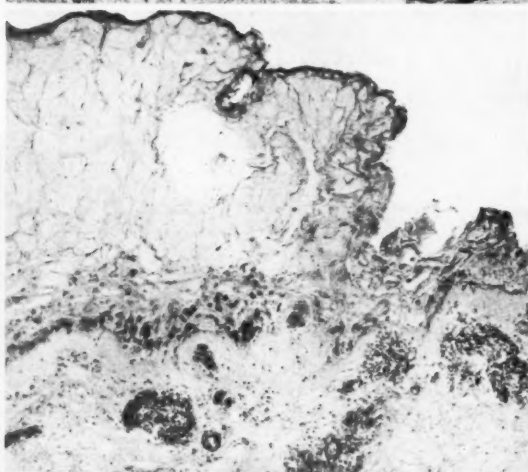
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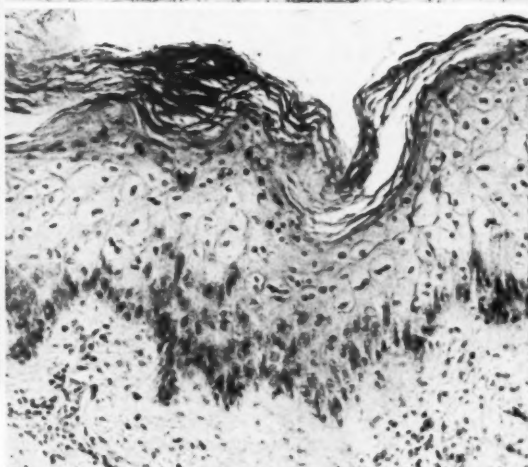
PLATE 162

Fig. 133. Regrowth of hair, following epilation. Patient had been approximately 1000 yds. from the explosion on the upper floor of a two-story Japanese building in Hiroshima. Epilation began on August 20, 2 weeks after the bombing. His white blood cell count had fallen to as low as 900 on September 4 and he had had fever, petechiae, and ulcerative and hemorrhagic gingivitis from which he recovered. Approximately 2½ months after the bombing. A.I.P. neg. HP 125.

Fig. 134. Group III. Scalp. Regenerating hair follicle to illustrate typical structure. Occasional mitotic figures among matrix cells. Well defined Henle's and Huxley's layers and external root sheath. Cuticle is forming. K-14. Yamamoto. Male, 25 years of age. Approximately 1000 yds. Died on the 47th day. A.I.P. neg. HM 210. × 225.

Fig. 135. Group II. Scalp. Atrophy of hair follicle, thickening of glassy and basement membranes. Failure of differentiation of internal root sheath. Irregularity of distribution of pigment. Vacuoles between epithelium and glassy membrane. K-45. Akagi. Male, 38 years of age. Approximately 1000 yds. Died on the 33rd day. A.I.P. neg. HM 238. × 130.

Fig. 136. Group II. Scalp. Hair follicle. Atrophy. Failure of differentiation of matrix. Irregularity of distribution of pigment. Tremendous thickening of glassy membrane. Remains of external root sheath have shrunken away from it. K-33. Ikeda. Male, 36 years of age. Approximately 1000 yds. Died on the 27th day. A.I.P. neg. HM 222. × 130.

133

134

135

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Pathology of Atomic Bomb Casualties

PLATE 164

FIG. 139. Group II. Scalp. Hair follicle. Atrophy. Hair remains fused to external root sheath. Process analogous to parakeratosis at surface. Irregularity of distribution of pigment. K-45. Akagi. Male, 28 years of age. Approximately 1000 yds. Died on the 33rd day. A.I.P. neg. HM 239. × 130.

FIG. 140. Group II. Hair. removed from head of partly epilated person. Tapering of shaft toward attached end; evidence of atrophy of matrix. Absence of remnants of internal root sheath. K-35. Takahashi. Male, 31 years of age. Approximately 1000 yds. Died on the 28th day. A.I.P. neg. HM 262. × 50.

FIG. 141. Group III. Scalp. Hair. (See also Fig. 38.) The complete follicle in longitudinal section. Atrophy of matrix. Failure of differentiation of internal root sheath. Irregular distribution of pigment. Thickening of glassy and connective tissue sheath. Remnants of atrophic shaft fill the dilated mouth of the follicle. Parakeratotic plug around this shaft. Atrophy of associated sebaceous gland. Other follicles are in process of regeneration (Figs. 134 and 142). K-14. Yamamoto. Male, 25 years of age. Approximately 1000 yds. Died on the 47th day. A.I.P. neg. HM 238. × 50.

FIG. 142. Group III. Regeneration of a hair is beginning at the base of the follicle by a renewed differentiation of matrix cells (in close apposition to the connective tissue papilla) and of the internal root sheath. From the same patient as Figure 141. A.I.P. neg. HM 208. × 115.

FIG. 143. Normal cycle of loss and replacement of a hair. At the left an external and an internal root sheath are still differentiated. At *b* the latter is no longer visible, and the former has become shrunken. There is a thickening both of the glassy and external cellular basement membrane. At *c, d,* and *e* there are successive stages of that process and the old hair is being extruded. At *f* the internal root sheath is being differentiated anew, and at *g* the new hair is pushing outwards in the old follicle. From Pinkus.[68] A.I.P. neg. HM 307.

139   140   141   142

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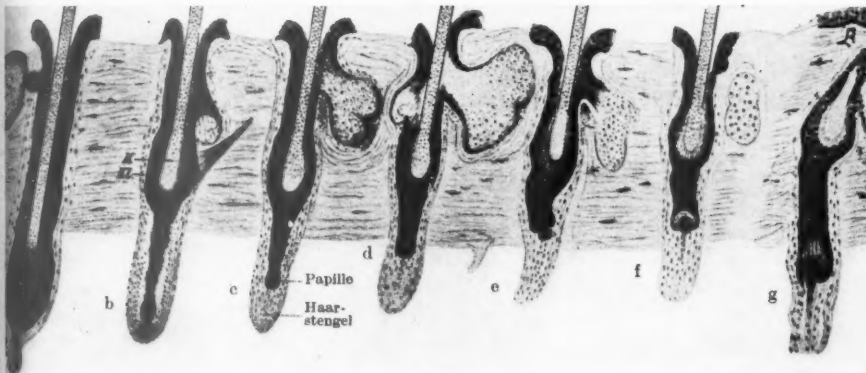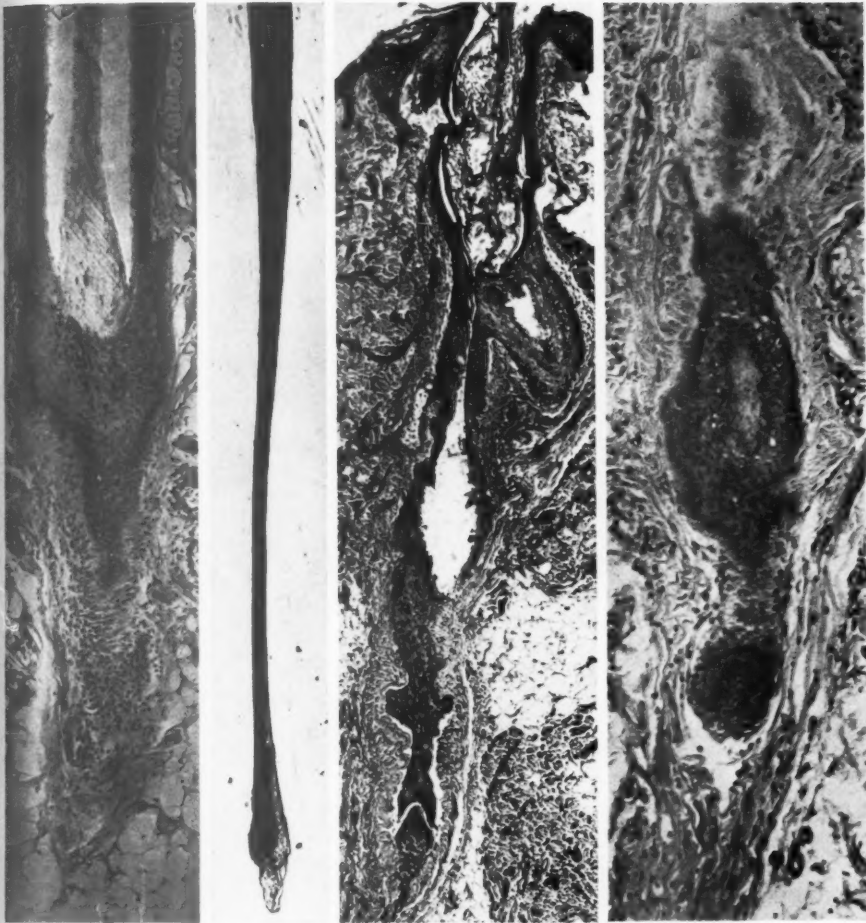
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THE PATHOLOGY OF HYPERPYREXIA
OBSERVATIONS AT AUTOPSY IN 17 CASES OF FEVER THERAPY[*]

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Heat in various forms is one of the oldest of therapeutic agents, although until recently it was employed only by local application.[1] Probably the first planned attempt to utilize increased body temperature as a therapeutic measure was made by Wagner von Jauregg in the malarial treatment of paresis.[2] Typhoid vaccine and other biologic substances have been used since, and various mechanical and electrical devices[3] have been constructed to serve the same purpose. Therapeutic hyperpyrexia has become an established means of treatment, not only for paresis, but for various forms of arthritis, chorea, asthma, sulfa-resistant gonorrhea, and other diseases.[4] As a consequence of its widespread adoption, numerous studies of the physiologic changes accompanying hyperpyrexia were undertaken. The results of some of these investigations are outlined briefly in the following paragraphs.

In the blood, hyperpyrexia produces leukocytosis; the red cell count and hemoglobin increase, the granulocytes exhibit a "shift to the left."[5,6] The sedimentation rate decreases,[5] and there is a reduction of the blood platelets.[7] Chemical studies show first a rise and then a fall of blood sugar.[6] Blood chlorides decline,[6] and values for nonprotein nitrogen[6] and lactic acid[8] are elevated. The plasma CO_2 content diminishes.[9,10] No changes are observed in values for calcium,[11] potassium,[11] or specific gravity.[12] Serum values for vitamin A decrease,[13] and there are conflicting reports regarding vitamin C.[14-16] Blood hormonal assays show increases of insulin, adrenalin, and of the antidiuretic hormone of the pituitary body.[17] Prothrombin[7,18] is diminished, and although there is no early change in plasma fibrinogen,[18] a late fall is observed. Miscellaneous findings include alkalinity of the urine and albuminuria[6]; both the heart and respiratory rates increase[5,19-21]; the blood pressure shows an early rise and late fall[5,19-21]; and counts of spermatozoa exhibit a reduction, lasting from 40 to 70 days.[22]

Experimentally, in rats, guinea-pigs, dogs, goats, and pigs,[23-27] the most common early changes are visceral congestion and disseminated focal hemorrhages in the internal organs; subendocardial hemorrhage is especially frequent. Dilatation of the right heart occurs and the blood is dark, fluid, and unclotted. There is pulmonary edema and

hemorrhage. Later (12 to 24 hours), parenchymatous degeneration is apparent in the adrenal cortex, liver, and renal tubular epithelium. Occasionally, lower nephron nephrosis is observed.

It was recognized early that fever therapy is not without danger, and rigid controls were adopted both in the selection of patients and in the method of administration. The Council on Physical Therapy of the American Medical Association, by means of a questionnaire circulated in 1934, ascertained that 29 deaths had occurred following pyretotherapy.[4] Accounts of at least 20 additional fatalities have since appeared in the literature,[28-33] mostly as sporadic case reports with inadequately described pathologic findings. Doubtless, deaths from this cause are far more frequent than indicated in the literature, yet comparatively few attempts have been made to study the morphologic changes which occur in human beings. Although the incidence of fatality in large series of cases is admittedly low,[34,35] complications are not uncommon and indicate more widespread deleterious effects than are usually attributed to this form of therapy. Clinically, jaundice occurs in from 14 to 19 per cent of the patients,[36] circulatory collapse and transitory neurologic changes in a significant percentage,[37] and evanescent electrocardiographic changes are almost invariable.[38]

The meager evidence in the literature does not permit an adequate understanding of the pathologic effects of hyperpyrexia. Nonspecific changes reported by various authors in their efforts to avoid overlooking significant lesions further confuse the situation. Clarification is possible only by study of a relatively large group of cases.

The pertinent material at the Army Institute of Pathology consists of the clinical records and autopsy protocols together with fixed tissues and stained sections from 21 cases of fatal therapeutic hyperpyrexia. Four of these were not included in this study: the clinical information was inadequate in 2, and in 2 others there were intercurrent diseases which had produced significant modifications. Of the 17 remaining patients (all males, 3 colored, 14 white, with ages ranging from 18 to 35 years) 15 received this form of therapy because of sulfa-resistant gonorrheal urethritis. One patient had nonsuppurative arthritis, type unspecified (case 16), and one, gonorrheal urethritis treated in 1936 before the advent of sulfonamide medication (case 14). In only 2 cases was there evidence of sulfonamide intolerance or sensitivity (cases 5 and 8) and in each of these the drug had been discontinued 1 month prior to fever therapy and subsequent death. The interval between the induction of hyperpyrexia and death varied from 3 hours to 14 days. In 5 cases, fever therapy had to be discontinued because of the develop-

CASE AND A.I.P. ACC.	HIGHEST FEVER (F)	DURATION OF FEVER (HOURS)	LENGTH OF SURVIVAL	SULFA DRUGS RECEIVED	TRANSFUSION	"SHOCK" DURATION	JAUNDICE	RENAL FAILURE	NEUROLOGIC CHANGES	R B C	W B C	BLOOD SUGAR (mgm%)	N P N (mgm%)	ICTERUS INDEX	CO₂ CONTENT (VOL%)	CREATININE (mgm%)	ALB	RBC	SEROUS EFFUSION	NON-FATTY VACUOLES	FATTY VACUOLES	CENTRAL NECROSIS	REGENERATION	PROXIMAL TUBULAR DEGENERATION	LOWER NEPHRON NEPHROSIS	EDEMA	NEURON DAMAGE	FATTY DEGENERATION	GRANULAR & HYALINE DEGENERATION	EDEMA
TREATMENT					CLINICAL						LABORATORY								URINE		PATHOLOGIC — LIVER				KIDNEY			BRAIN	HEART		
1 — 102904	106.5	3	3 HRS	+	-														PLEURAL 150cc R 150cc L	+	+			+		++			+	
2 — 99835	107.6	3	3 HRS	+	-															+	+			+	++	++	+			
3 — 93390	106	8	8 HRS	+	-				RESTLESS IRRATIONAL											+	++	?		++		++	++			
4 — 8251	106.6	8½	8½ HRS	+	-				STUPOROUS											++	+			+		++	++	++		
5 — 25072	106.8	6	11 HRS	+	-													NOT EXAMINED		+	+		+	+		+	++		+	
6 — 63533	106.7	11	11 HRS	+	PLASMA ONLY	½ HR			PUPILS UNEQUAL RESPIRATION											++	++			++	+	+	+			
7 — 93595	105	11	16 HRS	+	-	6 HRS			UNCONSC. PUPILS DILATED								++++			++	++		+	++		+	++		+	
8 — 100069	106	8	16 HRS	+	+	?	++	++			64	75	41			++++			+++	++	+	++	++	++	+	++		+	
9 — 99505	105.2	7	17 HRS	+	+	6 HRS	++	++			96.2	100	29-49	3.6	7.1	+++			+++	++		+++		++	+	+			
10 — 99501	107.8	3	20 HRS	+	-						36					++++				++	++					++		+	
11 — 100656	106	7	49 HRS	+	+	6 HRS													+++	+++	++++		+++			++			
12 — 100915	106.8	?	67 HRS	+	+	11 HRS	++			5.9 M	100		41			++		PLEURAL R.L. 1000cc L 1000cc R	++	++++	++++	?	+++	+	+	++	++	+	
13 — 99671	105	6-7	73 HRS	0	+	33 HRS	++		TREMORS OF ARM AND LEG	5.9 M 96% POLYS	45500 75% POLYS	152	15	38	1.3	++			+	++	++++		+++	+	+	++	++	+	
14 — 51838	107	5	93 HRS	0	+	33 HRS	++			4.9 M	11,000 75% POLYS	33	100	29-49	3.6	+++		PLEURAL 300cc R 300cc L	++	++++	++++	?	+++	+	+	++	++	+	
15 — 99594	108	6	4 DAYS	+	+	18 HRS	+++	++		4.15 M	9,000	154	109			+++		PLEURAL 150cc R 150cc L	+	++++	++++	++	+++	+	+	+++	++	+	+
16 — 99806	109	?	7 DAYS	0	+	?	+++	++	VACCINE VISIT SUT. CNS.	25,000	932	100	35			++++			++	++++	++++	+		+	+	+++		+	
17 — 30965	108	4	14 DAYS	+	+	18 HRS	++	+++		5.4 M		308	90			+		PLEURAL 150cc R 150cc L	+++	+++	+++	++	+++	++	++	++		+	

* RECEIVED INTRAVENOUS GLUCOSE

TABLE I
Clinical and Pathologic Findings in 17 Cases of Hyperpyrexia

ment of such untoward symptoms as shock, coma, convulsions, or hyper-excitability. The remaining deaths occurred in cases in which symptoms developed from 2 to 36 hours following a fever treatment lasting from 3 to 11 hours. Fever had been induced by the Kettering Hypertherm Cabinet (13 cases), intravenous typhoid vaccine (3 cases), and hot baths (1 case), but the pathologic changes found at autopsy did not differ with the method of heat induction. The height of the fever and the duration of treatment are listed in Table I.

CLINICAL FEATURES

All of the patients had been judged physically capable of enduring the treatment. The screening procedure included a complete physical examination, roentgenogram of the chest, blood count, chemical analysis of the blood, urinalysis, and electrocardiograms. Thirteen of the 17 patients were given a half-hour trial treatment which was well tolerated in each instance.

During the course of fever therapy all patients showed an increase in the pulse rate (110 to 160 per minute) and in the respiratory rate (32 to 46 per minute). Respirations were shallow, and slight to marked cyanosis was observed. The blood pressures fell somewhat following an initial transitory rise. Most patients vomited watery material one or more times. In 5 cases it was necessary to discontinue therapy because of the advent of coma, convulsions, circulatory collapse, or irrationality.

Circulatory collapse occurred in 9 cases. In 3, shock did not supervene until 2 to 36 hours had elapsed following completion of pyretotherapy. Clinical symptoms of pulmonary edema were present in 6 cases and of cerebral edema in 1 case (case 7).

Icterus occurred uniformly in all patients surviving longer than 49 hours. In the 3 who survived 4, 7, and 14 days, respectively, urinary suppression, hypertension, azotemia, and other clinical features of renal failure appeared before death.

LABORATORY STUDIES

Laboratory studies were recorded in only a few cases. The results are presented in Table I.

MORPHOLOGIC OBSERVATIONS

The initial effects of hyperpyrexia are clearly metabolic and after early death there is little that can be seen in the tissues. With time, however, progressive morphologic changes occur which give evidence of relatively irreversible functional injury to the cells.

General

Perhaps the most common feature at autopsy was the presence of moderate to marked congestion of all viscera and the brain. Congestion was present grossly in 15 cases and microscopically in all. It was most pronounced in the early cases (3 to 10 hours post-hyperpyrexia), being described by the prosectors as "marked" or "extreme," and diminished with time, so that in the cases in which survival exceeded 3 days, congestion was "slight" on gross examination or evident only microscopically.

Hemorrhages constituted another striking feature at autopsy and were present in all but 2 of the cases (Figs. 9 and 10). Their approximate incidence in various locations is shown in Table II.

TABLE II

The Incidence of Hemorrhage in 17 Fatal Cases of Induced Hyperpyrexia

Heart................................	17	*Gastro-intestinal tract*...............	7
Subepicardial....................	10	Esophagus........................	2
Subendocardial..................	5	Stomach.........................	3
Myocardial......................	2	Duodenum.......................	2
Brain................................	4	*Kidney pelvis*.......................	4
Pleura.............................	3	*Miscellaneous* (Skin, mucous membranes of mouth, sclera, mesentery, perirenal fat)....................	5

The hemorrhages, for the most part, were petechial, but in 2 cases they were ecchymotic, and in one the hemorrhagic areas in the skin measured as much as 5 cm. The frequency with which the heart was involved is corroborated by the experience of others.[32,39] One author[32] postulated that death in many of these cases was due to destruction of the conducting system of the heart by hemorrhage, a thesis which receives no support from our observations.

Transudates into one or more serous cavities occurred in 5 cases, in 4 of which survival exceeded 3 days. Pleural effusions of yellow serous fluid occurred in all 5; they were more voluminous on the right side and ranged from 150 to 1200 cc. Ascites amounting to 2200 cc. occurred in case 17; the patient had survived hyperpyrexia for 14 days. Pitting edema of the legs was present in 2 patients (cases 15 and 17), both of whom had shown clinical evidence of renal failure. Excess pericardial fluid was not observed in any case.

Jaundice was evident invariably in patients (cases 12 to 17) surviving longer than 49 hours. Its occurrence was directly related to the extent of liver damage and will be discussed when the pathologic changes of that organ are described.

Intravascular thrombosis, reported by others,[39] was not observed. On

the contrary, in 3 instances the prosector commented on the persistent fluidity of the blood and the almost complete absence of clotting.

Marked sicklemia occurred in 2 (cases 1 and 10) of the 3 Negro patients in this series. Neither patient had anemia or a clinical history suggestive of the sickle cell trait.

Liver

The hepatic changes were the most striking of those observed. Their relation to the length of survival substantiates the assumption that the effects of hyperpyrexia are essentially physiologic and metabolic initially, and that it is only with time that morphologic alterations occur.

The livers in early cases were described as normal or congested, but in cases in which survival was as long as 8 hours post-pyretotherapy, the prosectors noticed "mottling" of the parenchyma with pale pink or gray areas. The size of the "mottled" areas increased with survival time; simultaneously the organ became less firm. The average weight of the liver when death occurred rapidly was 1850 gm. When the patient had survived 72 hours or longer, the liver was grossly enlarged (average weight, 1975 gm.), extremely soft, and uniformly yellow.

Microscopically, the earliest changes (3 hours), aside from congestion, were loss of glycogen and "cloudy swelling" of the liver cells. Cytochondrial swelling of the type described by Opie[40,41] was observed (Fig. 1). At 8 hours post-hyperthermia, tiny vacuoles appeared in the cytoplasm (Fig. 2), particularly in the cells of the centrolobular areas. The majority of the small vacuoles did not take the usual stains for fat and within many of them there were small eosinophilic inclusions, 2 to 5 μ in diameter. (This phenomenon of "watery vacuolization" has been produced experimentally in animals subjected to anoxia and increased hepatic venous tension.[42]) By 10 hours, small fatty droplets appeared in the cytoplasm (Fig. 3). These coalesced and became larger, obscuring the small non-lipid vacuoles still present. An occasional specimen showed nuclear vacuolization, but the nature of these vacuoles has not been determined. With further survival, liver injury became more manifest and necrosis was observed in the centrolobular zone. At 16 hours this was minimal but by 60 hours some 60 per cent of the central part of the lobule was necrotic (Fig. 4) and hyperemic. If survival was sufficiently prolonged, reparative changes became increasingly prominent. At 7 days, phagocytosis of cellular detritus by macrophages was conspicuous (Fig. 6); polymorphonuclear leukocytes played only a minor rôle in this process. The relatively undamaged cells at the periphery of the lobule underwent active regeneration as evidenced by mitotic figures,

multinucleated liver cells, nuclear enlargement, and prominent nucleoli (Figs. 5 and 8). Proliferation of biliary channels occurred simultaneously. Morphologically, such a liver may resemble those found in fatal cases of epidemic hepatitis,[48] but the sparsity of inflammatory cellular reaction and the lack of liver atrophy are helpful differential features. Since the liver damage in fatal cases is presumably greater than that sustained by survivors of pyretotherapy, changes of this magnitude would not be expected among the latter. No appreciable fibrosis was present even in the case of longest duration (14 days) (Figs. 7 and 8).

Jaundice occurred only in patients surviving longer than 49 hours; in each of these the centrolobular destruction involved 40 per cent or more of the liver lobule.

Central Nervous System

The brain was examined in 16 cases; weights ranged from 1260 to 1700 gm. and averaged 1480 gm. Conspicuous congestion of the blood vessels of the meninges or underlying brain was recorded in 13 instances, edema was considered a feature in 9, and a cerebellar pressure cone in 1. Petechiae or small ecchymotic areas were present in 4. They involved the corpus callosum, the caudate nucleus, the periventricular region (Fig. 10), and the white matter of the cerebral cortex in close proximity to the gray matter. Focal subarachnoid hemorrhages occurred over the cerebellar hemispheres.

Microscopically, perivascular edema (Fig. 11) was present in the majority of the brains and "ring" hemorrhages in 4 (cases 3, 12, 15, and 16).

The parenchymal damage incurred by the central nervous system was most striking and constant in the cerebellum. When death occurred in less than 24 hours, focal neuronal degeneration was present in the Purkinje cell layer (Figs. 9 and 14), but the molecular and granular layers were not appreciably altered. With survival beyond 24 hours, degeneration was progressively more severe and was featured by edema and moderate reactive glial proliferation. In the patients who died later than 7 days after pyretotherapy, most of the Purkinje cells had disappeared and the few remaining were deeply stained and pyknotic. The dentate nucleus showed neuronal changes similar to those in the cortex which are described in the following paragraph.

Changes in the nerve cells of the cortex were present even in the earliest cases, of 3 hours' duration. In all cases the alterations were widely scattered and affected small focal areas. While the majority of the neurones were well preserved in these, some were swollen, exhibiting chromatolysis and karyolysis, and others were shrunken, with deeply

staining eosinophilic cytoplasm and pyknotic nuclei (Fig. 13). At this stage there was no apparent reaction on the part of the glia. Subsequently, disappearance or disintegration of nerve cells was observed in the focally damaged areas (Figs. 14 and 15). Glial reaction was not observed in the cerebrum or brain stem; presumably degenerative changes were too sparse and too widely scattered. In case 10, in which small vessels were occluded with masses of sickled red cells, definite areas of ischemic necrosis were most prominent in the deeper layers of the cerebral cortex and in the periventricular part of the thalamus (Fig. 12).

Similar but less severe neuronal changes were present throughout the basal ganglia and brain stem. No sections of spinal cord were available for study, but cellular damage in a fortuitously sectioned perivesical autonomic ganglion in one case suggested that the changes in nerve tissue might be widespread.

In 2 cases (10 and 12) the leptomeninges exhibited a very slight pleocytosis, the result of activation of histiocytes of the arachnoid trabeculae. According to Haymaker,[44] "similar changes have been observed in a great variety of disorders including anoxia of various types and toxi-infectious states."

Kidney

Renal changes were less constant than those already noted in the brain and liver. The kidneys were regarded as normal in those patients who died within 24 hours. Beyond this period, they were increased in weight, averaging 282 gm. each, and were described as pale, swollen, and somewhat softened. Petechiae were present beneath the pelvic mucosa in 4 (8 to 98 hours' duration) and in the perirenal fat in one.

Microscopically, congestion was marked if death occurred in less than 24 hours and slight interstitial hemorrhage was observed in one of these cases. With longer survival this feature was obscured by the more pronounced parenchymal changes. Parenchymatous damage was scanty in cases terminating rapidly. In the 7 cases in which death occurred within 12 hours, cloudy swelling of the tubular epithelium was the only consistent change and in places the swollen granular tubular cells occluded the lumen. In 2 of the 4 cases of 12 to 24 hours' duration, widespread karyolysis, karyorrhexis, and fatty degeneration involved the proximal convoluted tubules. Granular débris in the tubules appeared to have been derived from disintegration of the lining cells. Although absent in the early cases, in 5 of the 7 cases in which death was delayed for more than 48 hours the changes of lower nephron nephrosis[45] were apparent (Fig. 17). Pigmented casts were present within the lumina of distal convoluted tubules which showed progressive disintegration and detach-

ment of the lining epithelium. Concurrently, interstitial edema and cellular infiltration (largely lymphocytic) appeared. Numerous hyaline casts indicated a high degree of albuminuria and tubular stasis. Interstitial infiltrates were observed also in 2 of the earlier cases in which more widespread lesions indicated sulfonamides as the causative factor.[46,47] Three patients who survived 4, 7, and 14 days, respectively, died of renal failure with hypertension, azotemia, and oliguria.

Heart

The majority of the hearts in this series fell within the normal limits of weight; 2 showed an unexplained, probably pre-existent, increase to 460 and 510 gm. In 3 cases there was dilatation of the right auricle and ventricle. Hemorrhages were common (Table II) and occurred with equal frequency in the cases of short and long duration. The hemorrhages were petechial and occurred most commonly in the subepicardial tissues at the base of the heart. In 4 cases there were hemorrhages in the subendocardial layer of the interventricular septum, but microscopic examination revealed no involvement of the conduction bundles.

The microscopic changes in the heart were inconstant and, while more common in the cases of longer duration, were present also when survival was less than 24 hours. Focal degenerative myocardial changes were observed in 9 hearts: they were granular and hyaline in 5 (Fig. 18), fatty in 2, and lesions of both types were present in 2 others. Three hearts were the seat of slight stromal hyperplasia and cellular infiltration unrelated to the muscle lesions. These were considered results of sulfonamide administration, since infiltrates were present also in other organs.[46,47] Interstitial edema unrelated to renal failure occurred in 4 cases, fragmentation and rupture of muscle fibers in 2.

Lungs

The lungs usually were heavy, weighing together as much as 2560 gm. with an average weight of 1575 gm. In only one instance were the lungs of normal weight; in the others they were filled with edema fluid and blood. There were petechiae beneath the pleura in 5 cases. Microscopic examination merely substantiated the gross findings of edema, hemorrhage, and congestion. The changes of terminal bronchopneumonia were present in 2 cases.

Spleen

The spleen usually was enlarged, the average weight being 270 gm.; the range was from 160 to 530 gm. The spleen was soft and flabby with

an intensely congested, frequently diffluent pulp. In one case with sick-lemia the perifollicular hemorrhages reported by Rich[48] were observed.

Adrenals

Grossly, the adrenals were unaltered except for post-mortem autolysis of the medulla in a few cases. Microscopically, engorgement of the cortical sinusoids was obvious in cases of early death. The earliest paren-chymatous change was in the lipoid of the adrenal cortex. Normally, in the fasciculate zone, intracellular lipoid is present in the form of tiny uniform droplets, but as early as 3 hours after fever induction it was noted that these droplets had coalesced, appearing as large irregular vacuoles in the histologic sections (Fig. 20). The vacuoles increased in size pro-gressively until the cells disintegrated (Fig. 21). The loss of these cells and their replacement by fluid resulted in the formation of "tubular" structures similar to those reported by Rich[49] in fulminant infections (Figs. 22 and 23). In cases in which survival exceeded 24 hours, the cortical cells had a homogeneous eosinophilic cytoplasm without lipoid. Small foci of acute necrosis in the cortex were evident in 3 of the cases of 1 to 3 days' duration. This "tubular" change was no longer exhibited in any of the 5 which had a survival period of more than 3 days after fever therapy, suggesting that it is a form of reaction to an acute injury.[50] The adrenal medulla appeared normal in every case, but the other changes, although nonspecific, contradicted the statements by others[39] that no significant injury to the adrenals is caused by hyperthermia.

Testis

Gross abnormalities of the testes were not observed, although the prosector in one case described "diminished consistency." Microscopic changes, not present if death occurred within 8 hours, were noted fre-quently. Spermatogenesis was greatly decreased and assumed an abnor-mal pattern. Giant multinucleated cells were formed in the walls of the tubules (Fig. 24) and subsequently found their way into the lumina (Fig. 25). Such cells are not a specific effect of heat injury since they have been reported in deficiencies of vitamin A or E and in inanition.[51-53] It seems reasonable to regard them as abnormal forms resulting from the failure of cytoplasmic cleavage to keep pace with nuclear division. With progressive impairment of spermatogenesis (Fig. 26), the testicular tubules may consist solely of Sertoli cells. In the majority of cases the intertubular stroma was edematous but the interstitial cells were un-affected and there was no evidence of inflammatory infiltration. Whether recovery of spermatogenesis occurred could not be ascertained in these

cases. A clinical study, however, indicates that the spermatozoa counts return to normal in from 40 to 70 days following hyperpyrexia.[22]

Other Organs

Gastro-intestinal Tract. Hematemesis had been observed in 3 patients prior to death, but the hemorrhages encountered in 7 cases at autopsy were punctate and confined to the mucosa (Table II). Edema of the submucosa was noted occasionally and congestion was prominent in virtually all cases. Ulceration of the esophagus was present in 2 cases; one of these patients had been subjected to repeated passage of a stomach tube (case 14).

Bone Marrow. Histologic study of bone marrow was limited to 5 cases and none of these showed essential alteration of hematopoiesis. In one case of sicklemia (case 10), foci of fat necrosis occurred in the bone marrow (Fig. 19). In view of the limited nature of the material, the absence of changes must not be regarded as a denial of injury to megakaryocytes as reported in heat stroke.[54]

Sections of *pancreas, thyroid gland, skeletal muscle, urinary bladder,* and *prostate* contained no significant lesions that might be attributed to hyperpyrexia.

A summary of the more important clinical, laboratory, and pathologic findings is presented in Table I.

DISCUSSION

In fatal febrile conditions, it is impossible to segregate the morphologic effects of fever from those of the underlying process. As a consequence, the pathologic changes have never been clearly defined, although clinicians have long been aware of the danger of high body temperature, one of the commonest symptoms of disease. It seems particularly important, therefore, to report the observations in these 17 fatal cases which, although death was accidental, illustrate essentially the morphologic changes of controlled hyperpyrexia unmodified by other disease processes.

The explanation of the widespread effect of hyperpyrexia involves consideration of several factors. Kopp and Solomon[55] regarded shock as the sole pathogenic factor, and it is true that hemorrhages, serous transudates, focal myocardial degeneration, centrolobular hepatic necrosis, tubular degeneration and necrosis in the adrenal cortex, and lower nephron nephrosis have all been observed. But circulatory collapse occurred in only 9 of the 17 cases which we have studied. Moreover, their thesis merely substitutes one question for another, since the cause of

shock itself remains unexplained. We subscribe to Hartman's[56] view that anoxia constitutes the prime (but not the sole) injurious factor in hyperpyrexia, although we recognize that it may also occur in shock. Circulatory collapse, when present, serves only as an augmenting factor and adds anoxia of the stagnant variety to the anoxia already present. Hartman noted the similarity of the pathologic lesions following fever therapy and those due to prolonged asphyxia, as in carbon monoxide or nitrous oxide poisoning. He demonstrated experimentally that severe anoxia was produced constantly in animals by induced fever. Although oxygen determinations of the blood were not made in this series, the existence of significant anoxia was indicated by the constant occurrence of cyanosis, clinically, and by the presence of sickled red cells in the tissues at two of the post-mortem examinations. Since the routine laboratory studies had failed to reveal evidence of the sickle cell trait during life, we must infer a severe and prolonged anoxemia to account for the unmasking of the inherent cellular defect.

It is estimated that a body temperature of 106° F. increases the metabolic rate by 50 per cent,[57-59] and oxygen utilization proportionately. Yet the physiologic mechanisms for furnishing oxygen operate at reduced efficiency. At increased temperatures the oxygen-combining capacity of hemoglobin is diminished. Alkalosis induced by the hyperpnea of fever[58-64] results in an increased stability of oxyhemoglobin and impairs the release of oxygen to the tissues. Finally, the increased rate of blood flow reduces the time available for oxygen transfer.[19,65] Actual measurements by Cullen, Weir, and Cook[66] have shown that arterial oxygen saturation is decreased by approximately 25 per cent (comparable to that attained by ascent to an elevation of 17,500 feet) while venous oxygen saturation is increased to a fairly high level, thus demonstrating both a decreased supply and decreased utilization or delivery of oxygen. These conditions are not altered by oxygen administration and it is apparent that such therapy can only ameliorate,[60] and not prevent, deleterious effects. It is known that anoxia increases capillary permeability greatly (fluid passes through capillary walls at four times the normal rate after 3 minutes of anoxia[67,68]), and it seems likely that this accounts for the non-lipoid vacuoles in the cells of the liver and other organs. Similar changes have been produced experimentally in rabbits and occur with great rapidity.[69] They have been observed also in human material following accidental death from anoxic anoxia under conditions precluding survival for more than fractions of an hour.[42,69-75] The hydrostatic pressure of the blood, of major importance initially, becomes less essential as increase of survival time permits degradation of cytoplasmic

constituents. The latter process must certainly be associated with increased osmotic activity. It is to be expected that the changes would be most striking in the central portion of the hepatic lobule, considering that cells in that position are most remote from the arterial blood supply. Although the mechanism by which anoxia causes neuronal degeneration is not known, the sensitivity of the brain to oxygen lack is well established. It is logical to believe that identical lesions occurring in hyperpyrexia and in anoxic anoxia[74,75] are produced by the same mechanism. The site of the anatomic changes in the brain, though, may vary with the type of anoxia; according to Haymaker[44] the globus pallidus sustains the most severe damage in carbon monoxide poisoning, whereas the striatum bears the brunt of the injury in cyanide poisoning. The implication is that the metabolic injury producing cellular damage is more complex than simple oxygen lack.

Less measurable but none the less real alterations presumably occur among the cellular enzymes to explain the accumulation of fat in liver cells, myocardial fibers, and renal epithelium. The unraveling of the mechanism of carbohydrate utilization within the past decade has permitted an insight into the importance of complex interlocking and interrelated enzyme systems in cellular (and body) metabolism. Since enzymatic reactions are characteristically sensitive to alterations in temperature and pH, prolonged fever may be expected to have some influence on these vital activities. If sufficient damage is sustained, cellular metabolism will no longer be possible and necrosis will occur. With slighter degrees of injury the defect may become manifest as inability of the cell to utilize a material or materials which it ordinarily metabolizes. Using the conventional histologic technics, fat is the most readily demonstrable of these substances although there is no assurance that it is the only one. It seems likely that the inability of spermatogonia to divide properly represents another type of cellular metabolic inadequacy. The impaired capacity of the liver to form prothrombin and fibrinogen[18] is clearly attributable to a "bottleneck" in the cellular "production line." In this way the hemorrhagic tendency and decreased coagulability of the blood are aggravated. Other factors contributing to the latter are the direct destructive effects of heat on prothrombin,[76] platelets, and megakaryocytes,[7,54] increased capillary permeability,[67] and diminished capillary resistance.[77]

It is somewhat more difficult to explain the occurrence of lower nephron nephrosis in some of these patients. Transfusions or sulfonamides, either of which may cause kidney lesions of this type,[45] were given singly or together. However, since the transfusions were all compatible

and reactionless and the other viscera showed no morphologic evidence of sulfonamide sensitivity, it is probable that the renal injury was directly related to hyperthermia. Lower nephron nephrosis in 19 cases of heat stroke was unassociated with significant hypotension in 6.[54] Anoxemia must certainly be implicated in its causation as well as the cellular metabolic derangements more directly related to fever. Degradation products from quantitatively significant tissue necrosis, especially of the liver, are present to impose an additional burden upon renal function. Shock, should it supervene under such circumstances, would further diminish the likelihood of the kidneys escaping unscathed.

Finally, the similarity of the changes described to those occurring in thyroid crisis,[78-82] the postoperative liver-death syndrome,[83] and heat stroke,[54] all conditions in which hyperpyrexia is a prominent feature, would suggest a common mechanism of injury.

SUMMARY

Seventeen fatal cases of therapeutic hyperpyrexia have been reviewed to ascertain the nature of any pathologic changes that might have occurred. The underlying disease in each instance was of a type not usually associated with more than local tissue changes. Therefore, except for the complicating factors considered in the text, morphologic alterations were regarded as the result of the controlled fever.

Congestion and purpuric hemorrhages were the rule. Patients who did not die within 48 hours after the fever also exhibited jaundice. Microscopically, necrosis of cells and other degenerative changes were observed in the brain, heart, liver, kidney, adrenal glands, and testes. The alterations, especially in the liver, were progressively more severe as the survival time increased. It is inferred that the absence of visible change when death is prompt merely expresses the limitations of current histologic technics.

Anoxia and deleterious effects of elevated temperature upon essential cellular enzymes and enzyme reactions are probably the essential factors in producing these pathologic effects. Fever in many conditions besides pyretotherapy may produce similar lesions.

REFERENCES

1. Paget, S. For and Against Experiments on Animals. H. K. Lewis, London, 1912, 344 pp.
2. Wagner von Jauregg, J. Gutachten der Wiener medizinischen Fakultät. *Jahrb. f. Psychiat. u. Neurol.*, 1917–18, **38**, 1–48.
3. Neymann, C. A., and Osborne, S. L. Artificial fever produced by high frequency currents; preliminary report. *Illinois M. J.*, 1929, **56**, 199–203.

4. Council on Physical Therapy. Hyperpyrexia produced by physical agents. *J. A. M. A.*, 1934, **103**, 1308–1309.
5. Bierman, W., and Fishberg, E. H. Some physiologic changes during hyperpyrexia induced by physical means. *J. A. M. A.*, 1934, **103**, 1354–1357.
6. Simon, J. F. Effects of hyperpyrexia on the human blood count, blood chemistry and urine. *J. Lab. & Clin. Med.*, 1935–36, **21**, 400–404.
7. Wright, D. O., Reppert, L. B., and Cuttino, J. T. Purpuric manifestations of heat stroke. Studies of prothrombin and platelets in twelve cases. *Arch. Int. Med.*, 1946, **7**'j, 27–36.
8. Ferguson, C., Sophian, L. H., and Buckholtz, M. Lactic acid. Changes of its concentration in the blood following hyperpyrexia. *Exper. Med. & Surg.*, 1945, **3**, 104–106.
9. Ferguson, C., and Buckholtz, M. Effect of hyperpyrexia on the pH figure of the blood. *Arch. Phys. Therapy*, 1941, **22**, 333–334.
10. Osborne, S. L. Effect of hyperpyrexia on the blood pH. *Arch. Phys. Therapy*, 1941, **22**, 17–20.
11. Paul, W. D., and Kemp, R. C. Variations in concentration of certain electrolytes of blood serum during induced hyperpyrexia. *Proc. Soc. Exper. Biol. & Med.*, 1940, **45**, 427–431.
12. Osborne, S. L. Hyperpyrexia and the specific gravity of blood. *Arch. Phys. Therapy*, 1941, **22**, 407–409.
13. Thiele, W., and Scherff, I. Der Serum-Vitamin A-Spiegel im Fieber. *Klin. Wchnschr.*, 1939, **18**, 1275–1277.
14. Osborne, S. L., and Farmer, C. J. Influence of hyperpyrexia on ascorbic acid concentration in the blood. *Proc. Soc. Exper. Biol. & Med.*, 1942, **49**, 575–578.
15. Daum, K., Boyd, K., and Paul, W. D. Influence of fever therapy on blood levels and urinary excretion of ascorbic acid. *Proc. Soc. Exper. Biol. & Med.*, 1939, **40**, 129–132.
16. Zook, J., and Sharpless, G. R. Vitamin C nutrition in artificial fever. *Proc. Soc. Exper. Biol. & Med.*, 1938, **39**, 233–236.
17. Michez, J. Contribution à l'étude des effets de la diathermie sur le fonctionnement des glandes endocrines. *Ann. et bull. Soc. roy. d. sc. méd. et nat. de Bruxelles*, 1938, Nos. 9–10 (Annales), pp. 97–130.
18. Wilson, S. J., and Doan, C. A. The pathogenesis of hemorrhage in artificially induced fever. *Ann. Int. Med.*, 1940, **13**, 1214–1229.
19. Bazett, H. C. Circulation in pyrexia. *J. A. M. A.*, 1931, **97**, 1271–1274.
20. Benson, S. Relative influence of external and body temperatures upon the heart. *Arch. Phys. Therapy*, 1934, **15**, 303–304; 308.
21. Altschule, M. D., and Freedberg, A. S. Circulation and respiration in fever. *Medicine*, 1945, **24**, 403–440.
22. MacLeod, J., and Hotchkiss, R. S. The effect of hyperpyrexia upon spermatozoa counts in men. *Endocrinology*, 1941, **28**, 780–784.
23. Moritz, A. R., Henriques, F. C., Jr., Dutra, F. R., and Weisiger, J. R. Studies of thermal injury. IV. *Arch. Path.*, 1947, **43**, 466–488. Henriques, F. C., Jr. Studies of thermal injury. V. *Ibid.*, 1947, **43**, 489–502.
24. Hall, W. W., and Wakefield, E. G. A study of experimental heat-stroke. *J. A. M. A.*, 1927, **89**, 177–182.
25. Jacobsen, V. C., and Hosoi, K. The morphologic changes in animal tissues due to heating by an ultrahigh frequency oscillator. *Arch. Path.*, 1931, **11**, 744–759.
26. Mortimer, B. Experimental hyperthermia induced by high frequency current. *Radiology*, 1931, **16**, 705–719.

27. Baldwin, W. M., and Nelson, W. C. The histologic effects produced in albino rats by high frequency currents. *Proc. Soc. Exper. Biol. & Med.*, 1928–29, **26**, 588–590.

28. Wilbur, E. L., and Stevens, J. B. Morbid anatomic changes following artificial fever, with report of autopsies. *South. M. J.*, 1937, **30**, 286–290.

29. Hartman, F. W., and Major, R. C. Pathological changes resulting from accurately controlled artificial fever. *Am. J. Clin. Path.*, 1935, **5**, 392–410.

30. Watts, F. Abstracts of Papers and Discussions, Fifth Annual Fever Conference, Dayton, Ohio, 1935, p. 75.

31. Chunn, G. D., and Kirkpatrick, C. L. Fatal results of artificial fever therapy; a case report. *Mil. Surgeon*, 1937, **81**, 281–287.

32. Wilson, G. The cardiopathology of heat stroke. *J. A. M. A.*, 1940, **114**, 557–558.

33. Bragdon, J. H. The hepatitis of hyperthermia. *New England J. Med.*, 1947, **237**, 765–769.

34. Owens, C. A. The value of fever therapy for gonorrhea. *J. A. M. A.*, 1936, **107**, 1942–1946.

35. Trautman, J. A. Hyperpyrexia; indications and complications with evaluation of results based on 5,500 fever sessions. *New Orleans M. & S. J.*, 1940, **92**, 630–637.

36. MacDonald, R. M. Toxic hepatitis in fever therapy. *Canad. M. A. J.*, 1944, **51**, 445–449.

37. Collins, R. T. Transitory neurological changes during hyperthermia. *Bull. Neurol. Inst. New York*, 1938, **7**, 291–296.

38. Vesell, H., and Bierman, W. The electrocardiogram in fever; changes in induced hyperpyrexia. *Am. J. M. Sc.*, 1936, **191**, 484–491.

39. Lichtenstein, L. Pathological changes following therapeutic hyperthermia. *Am. J. Path.*, 1939, **15**, 363–376.

40. Opie, E. L. Cytochondria of normal cells, of tumor cells, and of cells with various injuries. *J. Exper. Med.*, 1947, **86**, 45–54.

41. Opie, E. L. Normal structure and degenerative changes of the cytoplasm of liver cells and of tumor cells derived from them. *J. Exper. Med.*, 1947, **85**, 339–346.

42. Pichotka, J. Tierexperimentelle Untersuchungen zur pathologischen Histologie des akuten Höhentodes. *Beitr. z. path. Anat. u. z. allg. Path.*, 1942, **107**, 117–155.

43. Lucké, B., and Mallory, T. The fulminant form of epidemic hepatitis. *Am. J. Path.*, 1946, **22**, 867–945.

44. Haymaker, W. Personal communication.

45. Lucké, B. Lower nephron nephrosis. *Mil. Surgeon*, 1946, **99**, 371–396.

46. French, A. J. Hypersensitivity in the pathogenesis of the histopathologic changes associated with sulfonamide therapy. *Am. J. Path.*, 1946, **22**, 679–701.

47. Frist, T. F. Reactions to sulfonamide compounds; a review of 186 cases. *War Med.*, 1944, **5**, 150–154.

48. Rich, A. R. The splenic lesion in sickle cell anemia. *Bull. Johns Hopkins Hosp.*, 1928, **43**, 398–399.

49. Rich, A. R. A peculiar type of adrenal cortical damage associated with acute infections, and its possible relation to circulatory collapse. *Bull. Johns Hopkins Hosp.*, 1944, **74**, 1–15.

50. Selye, H. General adaptation syndrome and diseases of adaptation. *J. Clin. Endocrinol.*, 1946, **6**, 117–230.

51. Mason, K. E. Differences in testis injury and repair after vitamin A-deficiency, vitamin E-deficiency, and inanition. *Am. J. Anat.*, 1933, **52**, 153–239.

52. Mason, K. E. Testicular degeneration in albino rats fed a purified food ration. *J. Exper. Zoöl.*, 1926, **45**, 159–229.

53. Pappenheimer, A. M., and Schogoleff, C. The testes in vitamin E-deficient guinea-pigs. *Am. J. Path.*, 1944, **20**, 239–245.

54. Malamud, N., Haymaker, W., and Custer, R. P. Heat stroke. A clinicopathologic study of one hundred twenty-five fatal cases. *Mil. Surgeon*, 1946, **99**, 397–449.

55. Kopp, I., and Solomon, H. C. Shock syndrome in therapeutic hyperpyrexia. *Arch. Int. Med.*, 1937, **60**, 597–622.

56. Hartman, F. W. Lesions of the brain following fever therapy; etiology and pathogenesis. *J. A. M. A.*, 1937, **109**, 2116–2121.

57. Barr, D. P., Cecil, R. L., and DuBois, E. F. Clinical calorimetry. XXXII. Temperature regulation after the intravenous injection of proteose and typhoid vaccine. *Arch. Int. Med.*, 1922, **29**, 608–634.

58. Bazett, H. C. Studies on the effects of baths on man. I. Relationship between the effects produced and the temperature of the bath. *Am. J. Physiol.*, 1924, **70**, 412–429.

59. Landis, E. M., Long, W. L., Dunn, J. W., Jackson, C. L., and Meyer, U. Studies on the effects of baths on man. III. Effects of hot baths on respiration, blood and urine. *Am. J. Physiol.*, 1926, **76**, 35–48.

60. Wallace, J., and Bushby, S. R. M. Hazards of hypertherm treatment. *Lancet*, 1944, **2**, 459–464.

61. Wallace, J., and Bushby, S. R. M. Physiological and biochemical changes following hypertherm treatment. *Brit. J. Ven. Dis.*, 1943, **19**, 155–162.

62. Danielson, W. H., Stecher, R. M., Muntwyler, E., and Myers, V. C. The acid-base balance of the blood serum in hyperthermia. *Am. J. Physiol.*, 1938, **123**, 550–557.

63. Bischoff, F., Long, M. L., and Hill, E. Studies in hyperthermia. II. The acid-base equilibrium in hyperthermia induced by short radio waves. *J. Biol. Chem.*, 1931, **90**, 321–329.

64. Carryer, H. M. Tissue anoxia resulting from respiratory alkalosis. *Proc. Staff Meet., Mayo Clin.*, 1947, **22**, 456–464.

65. Kissin, M., and Bierman, W. Influence of hyperpyrexia on velocity of blood flow. *Proc. Soc. Exper. Biol. & Med.*, 1932–33, **30**, 527–530.

66. Cullen, S. C., Weir, E. F., and Cook, E. The rationale of oxygen therapy during fever therapy. *Arch. Phys. Therapy*, 1942, **23**, 529–535.

67. Simpson, W. Studies on the physiology of fever. *J. A. M. A.*, 1936, **106**, 246.

68. Moon, V. H. The vascular and cellular dynamics of shock. *Am. J. M. Sc.*, 1942, **203**, 1–18.

69. Trowell, O. A. The experimental production of watery vacuolation of the liver. *J. Physiol.*, 1946, **105**, 268–297.

70. Müller, E., and Rotter, W. Über histologische Veränderungen beim akuten Höhentod. *Beitr. z. path. Anat. u. z. allg. Path.*, 1942, **107**, 156–172.

71. Hesse, W. Untersuchungen über das Bild der vakuoligen Degeneration in der Leber am menschlichen Sektionsgut. *Beitr. z. path. Anat. u. z. allg. Path.*, 1942, **107**, 173–186.

72. Ladewig, P. Anoxaemic changes in the liver, with regard to the "high-altitude death" of airmen. *Nature, London*, 1943, **151**, 558.

73. Lewis, R. B., and Haymaker, W. High altitude hypoxia. *Aviation Med.*, 1948, **19**, 306–336; 354.

74. Kritzler, R. A. Acute high altitude anoxia; gross and histologic observations in twenty-seven cases. *War Med.*, 1944, **6**, 369–377.

75. Titrud, L. A., and Haymaker, W. Cerebral anoxia from high altitude asphyxiation. *Arch. Neurol. & Psychiat.*, 1947, **57**, 397–416.
76. Quick, A. J. The Hemorrhagic Diseases and the Physiology of Hemostasis. C. C Thomas, Springfield, Ill., 1942, 340 pp.
77. Rossman, P. L. Capillary resistance in artificially induced fever. *Clin. Bull., Cleveland Univ. Hosps.*, 1939, **3**, 89–91.
78. Farrant, R. Hyperthyroidism: its experimental production in animals. *Brit. M. J.*, 1913, **2**, 1363–1367.
79. Goodpasture, E. W. Myocardial necrosis in hyperthyroidism. *J. A. M. A.*, 1921, **76**, 1545–1551.
80. Youmans, J. B., and Warfield, L. M. Liver injury in thyrotoxicosis as evidenced by decreased functional efficiency. *Arch. Int. Med.*, 1926, **37**, 1–17.
81. Boyce, F. F. The Rôle of the Liver in Surgery. C. C Thomas, Springfield, Ill., 1941, 365 pp.
82. Ficarra, B. J., and Naclerio, E. A. Thyroid crisis; pathogenesis of hepatic origin. *Am. J. Surg.*, 1945, **69**, 325–337.
83. Sutton, J. E., Jr. Acute postoperative necrosis of the liver. *Am. J. M. Sc.*, 1936, **192**, 219–224.

DESCRIPTION OF PLATES

PLATE 165

FIG. 1. Case 1, Army Institute of Pathology accession no. 102884. Liver, demonstrating cellular granularity (cytochondrial swelling) after 3 hours of hyperpyrexia. × 435. Negative no. 103922.

FIG. 2. Case 3, A.I.P. acc. 93390. Non-lipid vacuolization of liver cells 8 hours after onset of hyperpyrexia. × 515. Neg. 103909.

FIG. 3. Case 8, A.I.P. acc. 10169. Large fatty and small non-lipid vacuoles of the liver cells. × 600. Neg. 103722.

FIG. 4. Case 15, A.I.P. acc. 89594. Centrolobular degeneration of liver 100 hours after onset of fatal hyperpyrexia. × 100. Neg. 74693.

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FIG. 5. Case 16, A.I.P. acc. 79906. Mitotic figures in liver showing regenerative activity 7 days after therapeutic fever. × 475. Neg. 103706.

FIG. 6. Case 16, A.I.P. acc. 79906. Focal accumulation of macrophages replacing liver cells. × 395. Neg. 103705.

FIG. 7. Case 17, A.I.P. acc. 130943. Considerable loss of liver cells, stromal collapse, and hyperplasia of the residual cells have occurred. × 70. Neg. 103925.

FIG. 8. Case 17, A.I.P. acc. 130943. From the same section as Figure 7, but at a higher magnification. Of note are the mononuclear character of the leukocytic response, the proliferating bile ducts, and the bile "thrombi." × 275. Neg. 103926.
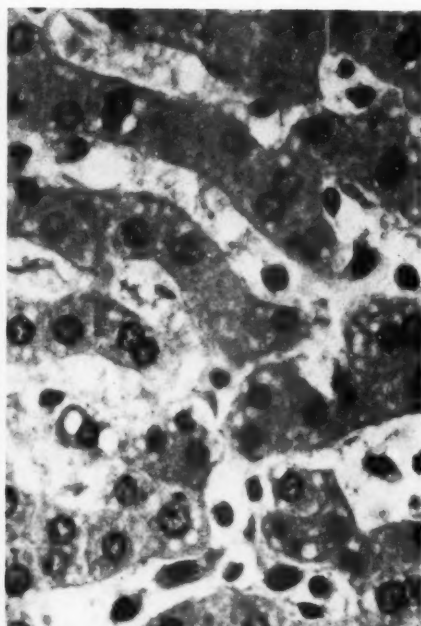
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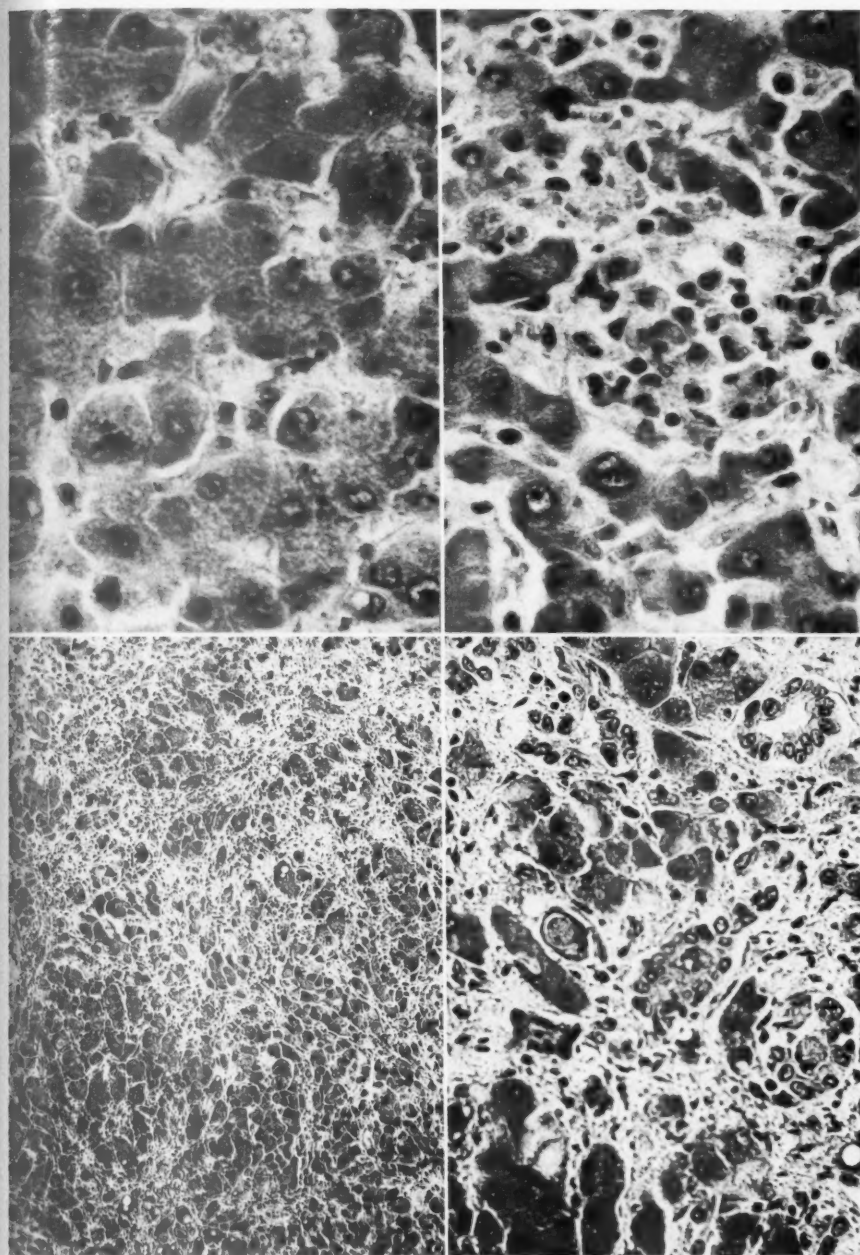
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PLATE 167

FIG. 9. Case 15, A.I.P. acc. 89594. Cerebellum 100 hours after fever therapy. Subarachnoid hemorrhage and degenerative changes are seen in the Purkinje cell layer. × 75. Neg. 103724.

FIG. 10. Case 15, A.I.P. acc. 89594. Hemorrhages beneath the subependymal cell plate of the third ventricle 100 hours after fever therapy. × 15. Neg. 74071.

FIG. 11. Case 17, A.I.P. acc. 130943. Cerebrum 14 days after fever therapy. There is perivascular edema of the subcortical white matter. × 75. Neg. 103711.

FIG. 12. Case 10, A.I.P. acc. 95801. Focal degeneration in the caudate nucleus 20 hours after artificial fever. Sicklemia had not been recognized during life. × 65. Neg. 103698.

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FIG. 13. Case 1, A.I.P. acc. 102884. Inferior olivary nucleus, medulla, 3 hours after the onset of artificial fever. A few of the nerve cells exhibit karyolysis, others are pyknotic. × 475. Neg. 103729.

FIG. 14. Case 7, A.I.P. acc. 93695. Purkinje cell layer of the cerebellum 12 hours after the onset of hyperpyrexia. There is loss of nuclear structure in all of the Purkinje cells in this field. × 515. Neg. 103716.

FIG. 15. Case 10, A.I.P. acc. 95801. Hippocampus 100 hours after fever, showing acute degenerative changes in the cells of Sommer's sector. × 275. Neg. 104346.

FIG. 16. Case 15, A.I.P. acc. 89594. Hypoglossal nucleus showing both cellular lysis and pyknosis 100 hours after artificial fever. × 355. Neg. 103735.
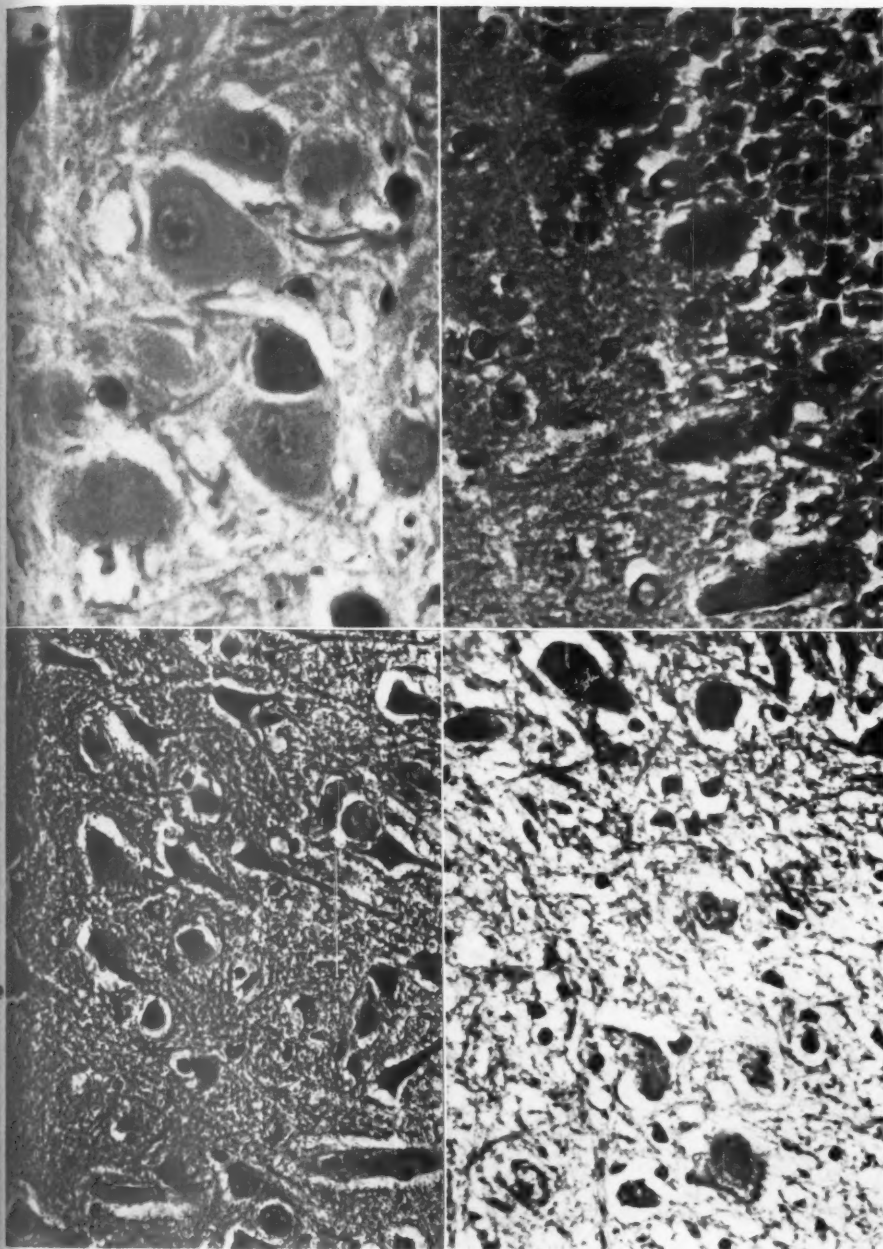
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FIG. 17. Case 15, A.I.P. acc. 89594. Distal nephron nephrosis 100 hours after onset of hyperpyrexia. × 350. Neg. 74686.

FIG. 18. Case 10, A.I.P. acc. 95801. Focal hyaline and granular degeneration of myocardium 20 hours after onset of hyperpyrexia. × 400. Neg. 103701.

FIG. 19. Case 10, A.I.P. acc. 95801. Focal area of fat necrosis in the bone marrow 20 hours after onset of hyperpyrexia. The sickle cell trait had not been recognized during life. × 150. Neg. 103695.

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Fig. 24. Case 4, A.I.P. acc. 82251. Seminiferous tubules 8½ hours after hyperpyrexia. Spermatogenesis is reduced. A giant cell form is visible within the wall of the tubule at the center of the field. × 187. Neg. 103733.

Fig. 25. Case 5, A.I.P. acc. 125672. Seminiferous tubule 11 hours after hyperpyrexia. Spermatogenesis is greatly reduced. Four giant cell forms are present within the lumen. × 275. Neg. 103717.

Fig. 26. Case 15, A.I.P. acc. 89594. Seminiferous tubule 100 hours after hyperpyrexia. Spermatogenesis past the stage of primary spermatogonia has ceased. The latter are greatly reduced in number. Of note is the intraluminal giant cell, the nuclei of which have the same characteristics as the adjacent spermatogonia. × 235. Neg. 103703.
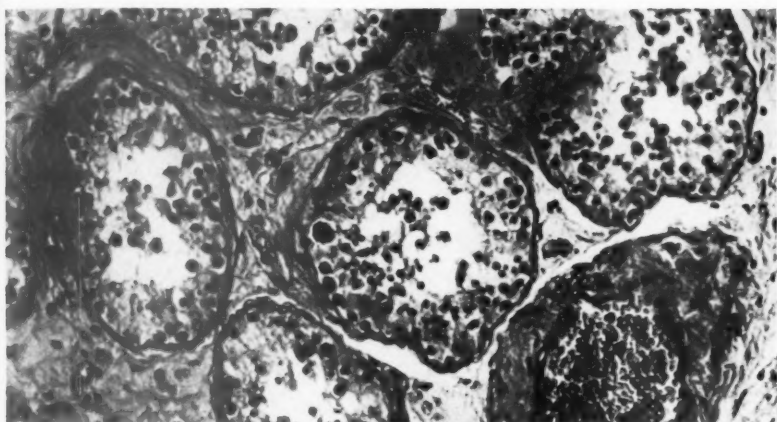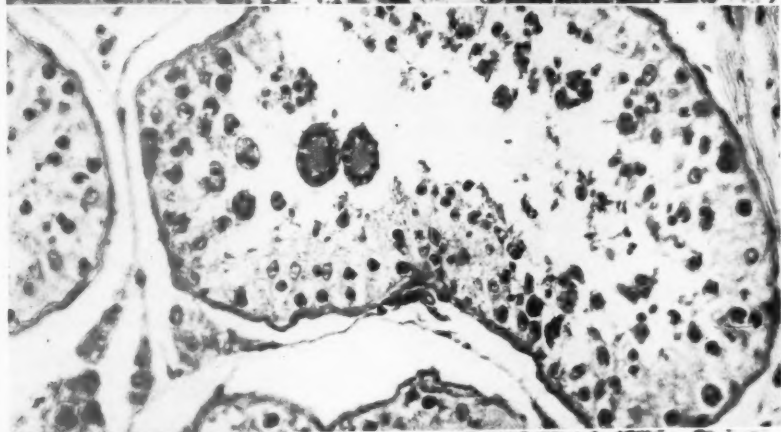
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PLATE 171

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Pathology of Hyperpyrexia

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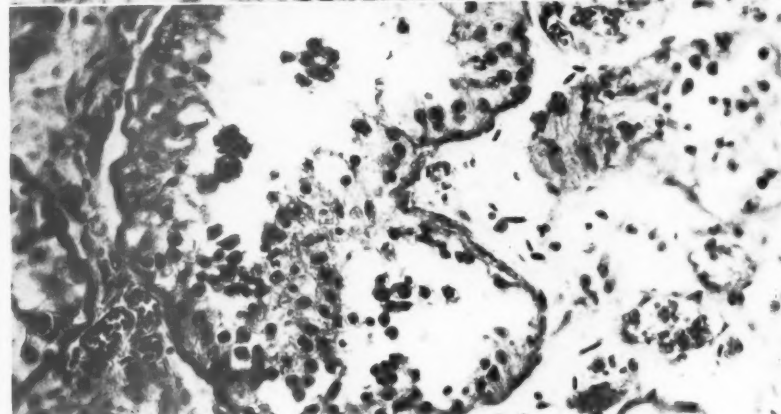
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HISTOCHEMICAL STUDIES ON THE PATHOGENESIS OF FIBRINOID*

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Disease processes that affect the connective tissues have been studied intensively during recent years. As in other tissue systems, it has become increasingly apparent that reactions of the connective tissue to injury follow generally prescribed patterns. One such pattern of alteration has come to be known as fibrinoid degeneration. This term, first used by Neumann,[1] is applied to the appearance of a homogeneous, eosinophilic, relatively acellular, refractile substance with some of the tinctorial properties of fibrin.

Those who have studied fibrinoid have presented a variety of ideas concerning its significance and pathogenesis. Fibrinoid was described in inflammations of the serous, mucous, synovial, and endothelial surfaces by Neumann.[1] He stated also that band-like structures in cysts, bursae, and ganglia gave identical staining reactions with picrocarmine or picrofuchsin. Ricker[2] described the process in hygromas and in certain tumors; Askanazy[3] referred to fibrinoid in the base of peptic ulcers; Klinge[4] believed that an alteration of this type was the primary change in rheumatic fever; and Schosnig[5] demonstrated it in a variety of specific and nonspecific inflammations. Fibrinoid degeneration has been described also in rheumatoid arthritis,[4,6] periarteritis nodosa,[7] scleroderma,[8,9] thrombo-angiitis obliterans,[10] disseminated lupus erythematosus,[11] malignant hypertension,[12] and arteriosclerosis.[13] Grosser[14] applied the term to the nonfibrous, homogeneous, acellular substance in the placenta. He distinguished the material from fibrin but did not believe it to be related to the fibrinoid occurring elsewhere in the body.

Fibrinoid has been successfully produced by a variety of experimental technics, including simple squeezing of tissue,[15] orthostatic hypotension,[16] acute bacterial infection,[5] prolonged passive hyperemia,[17] administration of large doses of desoxycorticosterone acetate,[18] and hyperergic inflammations.[19]

The theories proposed for the formation of fibrinoid include precipitation and inspissation of fibrin or other blood derivatives,[16,20] necrosis of collagen,[4,21] "coagulation" of the ground substance,[11,22] or a combination of these processes. This aspect of the problem has been reviewed by

Bahrmann.[21] With regard to the development of fibrinoid in the Aschoff nodule, it has been shown that the early stages consist of a mucinous edema (of Talalajew[23]) and then a fine fibrillar precipitate. The fusion into homogeneous bands and breakdown into granular masses are believed to be later stages in its development.[4,23,24]

Because fibrinoid degeneration is a connective tissue manifestation of allergic reactions, some investigators believe that any disease characterized by fibrinoid change is of allergic origin. Since this alteration may be produced by a variety of methods, however, it has been suggested that it is not specific for injury of any particular type but represents the limitations of the connective tissue response. Klemperer, Pollack, and Baehr,[11,22] Baehr and Pollack,[25] and Klemperer[26] have emphasized this point and have suggested that the cause of the manifest alteration be sought in the chemical and physical properties of the connective tissue. They pointed out, too, that the significance of the process may well lie in its influence on hematoparenchymal permeability or some other physiologic function of the connective tissue.

As a result of extensive investigations, chiefly by Duran-Reynals,[27] Meyer,[28] Clark and Clark,[29] Bensley,[30] Chain and Duthie,[31] and McClean,[32] it is now believed that the amorphous ground substance of the connective tissue has a gel-like structure because of its content of acid mucopolysaccharides. Hyaluronic acid, the chondroitin and the mucoitin sulfuric acids (heparin belongs in this latter group) are examples of this type of compound. The chemical and biologic features of these substances have been covered well in a series of articles.[27,28,33-35] It is desirable, however, to review briefly those properties which are germane to the development of our thesis.

Hyaluronic acid, for example, is a long-chain polymer whose constituent units are N-acetyl glucosamine and glucuronic acid. Because of its asymmetry and size, it is highly viscous in low concentrations. It will react with proteins on the acid side of their iso-electric point to yield a coprecipitate or coacervate. In alkaline solutions, in solutions of weak electrolytes, and in distilled water, structures rich in this substance are known to swell.[36] These characteristics, as well as the ability to induce metachromasia, to be discussed later, are shared by all acid mucopolysaccharides and are dependent in part upon a high degree of polymerization. Should the material be depolymerized, viscosity is reduced and ability to combine with protein and induce metachromasia is lost.

In addition to its occurrence in the amorphous ground substance of the connective tissue, hyaluronic acid occurs in the aqueous and vitreous

humors of the eye, synovial fluid, Wharton's jelly, and the material surrounding the ovum as it is extruded from the ovary. In some of these sites it occurs in combination with other substances of this type. Acid mucopolysaccharides, differing from hyaluronic acid, occur in the ground substance of the wall of the aorta and other blood vessels, in the ground substance of cartilage and tendons, in granules of certain cells, and as a component of epithelial mucins.

It was a premise of this investigation that the essential feature of fibrinoid formation is the precipitation of the acid mucopolysaccharide of the ground substance. If, indeed, this is the case, the precipitation could be brought about in various ways. For example, either the lowering of the tissue pH below that of the iso-electric point of the tissue proteins or the addition of an alkaline protein (on the acid side of the iso-electric point at normal body pH) might act as a precipitating factor. It is conceivable, too, that an abnormal protein (something akin to the C reactive protein) could be the precipitant.

Many phenomena related to fibrinoid formation, however, have been shown to be associated with an increase in tissue basicity. Knepper,[37] using a quinhydrone electrode, demonstrated that the Arthus reaction is accompanied by an increase in tissue pH. He was of the opinion that the degree of alkalinity could be correlated with the extent of necrosis. Serosal reactions, tuberculous infections, diphtheritic membranes, and granulation tissue also were found to be associated with an increase in tissue pH. Schade[38] was of the opinion that such alkalinity was due to the loss of carbon dioxide, but it has been calculated that such a mechanism alone could not account for the degree of change observed. Koller and Leuthardt,[39] investigating the caseous material in tuberculous cavities, emphasized the alkaline nature of the necrotic substances. Changes in the staining characteristics (increased eosinophilia, for example) and the isolation of alkaline substances from lesions induced by allergic injury[40-42] tend to support the above findings and suggest that the change in pH is due to the liberation of alkaline substances.

If, then, precipitation of the acid mucopolysaccharide gives rise to fibrinoid, it seems likely that the combination with an alkaline protein is the mechanism involved. The investigations reported herein were designed to test this hypothesis.

MATERIALS AND METHODS

Materials selected for study consisted of formalin-fixed tissue embedded in paraffin. The material studied is given in Table I. We are aware

that formalin is not the ideal fixative, but there was no choice in method. Limited experience with frozen sections and other more satisfactory methods of fixation[48] have supported and emphasized some of the changes to be described.

TABLE I

Materials Studied, Source and Number

Rheumatic fever	15	Dermatomyositis	1
Rheumatoid arthritis	10	Scleroderma	6
Periarteritis nodosa	5	Gastric ulcer	5
Disseminated lupus erythematosus	9	Placentae	5
Bursitis, "ganglia," synovitis,		Buerger's disease	5
tenosynovitis	16	Arteriosclerosis	3
		Total	80

Sections were cut at 6 to 8 μ and stained with 1 per cent toluidine blue, crystal violet, van Gieson's stain, and phosphotungstic acid hematoxylin after chromium mordanting.[44] The periodic acid leukofuchsin method, as used by McManus[45] and by Hotchkiss,[46] was employed to demonstrate polysaccharide aldehyde. Control sections for plasmal were run.[47] Desoxyribonucleic acid was demonstrated by the method of Feulgen as applied by Stowell.[48] On selected sections the Sakaguchi reaction for arginine as modified by Weber[49] and by Serra[50] was employed. When metachromasia was demonstrated with toluidine blue, representative sections were incubated with a 5 mg. per cc. of a 90 VRU (viscosity reducing unit) per mg. hyaluronidase (testis) preparation in saline solution for 1 hour at room temperature using saline and albumin in saline controls.

To facilitate interpretation of the results, the significance of the staining reactions is indicated briefly.

Periodic acid is known to free the aldehyde groups of polysaccharides[51] and has been used in combination with the Schiff leukofuchsin reagent in many histochemical studies. Hotchkiss[46] set forth the requirements for a substance to give a positive reaction. It must contain a 1,2 glycol grouping or its equivalent; it must not diffuse away during the process of fixation; it must give an oxidation product which is not diffusible and which will be present in sufficient amount to produce a detectable color. Under the conditions employed in this test it appears that only the polysaccharides and mucoproteins are likely to be present in amounts adequate to produce a visible reaction. It has been stated that ribonucleic and desoxyribonucleic acid, the hydroxyamino acids (with the possible exception of hydroxylysine), the carbohydrates low in molecular weight, the cerebrosides, and inositol-containing lipids will not give a positive

result either because the carbohydrate components are chemically substituted so that the free glycol grouping is not present or the materials are removed in the process of fixation. Hotchkiss demonstrated that the acid mucopolysaccharides will give a reaction in a spot test.

The significance of metachromasia with basic aniline dyes has not as yet been entirely explained. Lison[52] was of the opinion that this phenomenon is induced only by high molecular substances which contain ester sulfate linkages. Wiame[56] believed it to be demonstrated by all substances of high molecular weight with an acidic function. That this latter interpretation is closer to the real state of affairs can no longer be doubted at the present time. Kelley and Miller[53] demonstrated that certain nuclear components and silicic acid gels may be metachromatic. The observations of Wislocki and co-workers,[54,55] and of Meyer,[28] as well as our own experience, indicate that hyaluronic acid, which contains no sulfate groups, may also be metachromatic. Desoxyribonucleic acid, hexametaphosphate,[56] and a co-polymer of maleic anhydride and styrene will also induce this change.[57] Evidence is at hand which suggests that polymerization of molecules of the dye is associated with the development of metachromasia.[58] The conditions best suited for the development of metachromasia are not known, nor is it known why the various basic aniline dyes differ in their ability to demonstrate this phenomenon.

Hyaluronidase derived from testis cannot be regarded as being unequivocally specific for hyaluronic acid. Meyer[28] has shown that some chondroitin sulfuric acids of tissues and hyalurono-sulfuric acid are depolymerized by this preparation. It is easily demonstrated, however, that the metachromatic substances in the wall of the aorta and other blood vessels, cartilage, mast cells, and mucous glands are not affected by the preparation used in this investigation. Since the reaction with alkaline proteins is a general characteristic of acid mucopolysaccharides, this lack of specificity is not considered to be of serious consequence.

Serra[50] has claimed that the arginine reaction may be used to determine the basicity of a protein histochemically. This claim is predicated on the fact that the basic amino acids—arginine, histidine, and lysine—are present in increased amounts in alkaline proteins. Although fibrinoid contains considerable quantities of arginine, we have attempted to interpret the reaction with caution for, in general, the basicity of a protein depends on the relative quantities of basic and acidic (di-carboxylic) amino acids. Collagen which is not a particularly basic protein, for example, contains significant quantities of arginine.[59]

RESULTS

The following outline presents the usual staining reactions of fibrinoid:

    Toluidine blue   — may or may not be metachromatic
    Crystal violet   — purple
    Phosphotungstic acid
        hematoxylin   — yellow to yellow orange with a
                              superimposed blue fibrillar mate-
                              rial on occasion
    van Gieson's stain   — yellow to orange to red
    Periodic acid
        leukofuchsin   — red
    Sakaguchi[49,50]   — yellow orange

The relationships of fibrinoid in the various disease processes will be discussed separately.

Rheumatic Fever

Swelling of the endocardium (auricular, ventricular, and valvular) and subendocardium was associated with an increase of metachromatic material. In the early lesions most of the metachromasia could be prevented by prior incubation of the section with hyaluronidase. It is our impression that in older lesions the material was more resistant to the action of this enzyme. Chromatropic substances with an identical response to hyaluronidase incubation were noted in the pericardium.

In the valvular structures fibrinoid was present deep in the valve and at the surface. Before fibrinoid material was actually formed, the subendothelial swelling of the valve in rheumatic fever was associated with an increase of metachromatic material. In the older lesions which had ulcerated, the vegetation stained orange with phosphotungstic acid hematoxylin and there was a blue fibrillar material superimposed in areas. With the van Gieson technic the vegetation stained yellow, with toluidine blue it was light green, and with periodic acid it was aldehyde-positive. In most instances fibrin did not stain positively with the last-mentioned technic, but it was observed to do so when in close proximity to lesions involving the endothelium. It was noted also that streamers in thrombi and the cytoplasm of leukocytes might contain aldehyde-positive substances, and it is possible that these substances contributed to the staining reactions of the vegetation.

The material deep in the valve structures stained orange with phosphotungstic acid hematoxylin, and brilliantly red with leukofuchsin after periodic acid oxidation; it did not ordinarily stain metachromatically

with toluidine blue but was surrounded by metachromatic material. With the van Gieson technic we observed the usual reaction for fibrinoid but also noted that substances which undoubtedly were fibrinoid might stain red. Hyaluronidase, at times, reduced the metachromasia in the valve substance (Figs. 1 and 2).

An increase of the metachromatic substance also occurred interstitially and in the region of Aschoff bodies. In the Aschoff body the fibrinoid structure might or might not stain metachromatically with toluidine blue. Metachromatic material was invariably in close proximity with fibrinoid, however, and had the same configurational distribution. With the periodic acid leukofuchsin reagents, fibrinoid was aldehyde-positive. With the van Gieson stain, an admixture of yellow and red fibers, continuous in some areas, was noted, confirming the findings of Gross and Ehrlich.[60] That portion of the nodule staining metachromatically appeared red with van Gieson's stain, while the fibrinoid appeared yellow or orange. No fibrin was demonstrable with phosphotungstic acid hematoxylin. In the nodule, too, hyaluronidase incubation studies indicated that the material present in early lesions was sensitive to the action of the enzyme, whereas the metachromatic material in older lesions was somewhat more resistant (Figs. 3 and 4).

Chromatropic material was noted in increased quantities in the coronary vessels and in the nerves. In the vessels, chromatropic edema, sclerosis, and fibrinoid formation were noted in all layers. The findings, however, were essentially in agreement with those of Karsner and Bayless[61] in that chromatropic edema and fibrinoid formation seemed to be more common in the media. The metachromatic substances in the intima and media were resistant to the action of hyaluronidase. The mucoid accumulation in the nerves, similar to the alterations described by Fröhlich,[62] Lasowsky and Kogan,[63] Schultz,[64] and Krücke,[65] was sensitive to the action of hyaluronidase.

Throughout the heart, with the exception of the walls of the larger blood vessels, increased numbers of mast cells were encountered.

Rheumatoid Arthritis

In the subcutaneous nodules from cases of rheumatoid arthritis the greatest concentration of the metachromatic material was in the stellate zone. In formalin-fixed sections the fibrillar material in this zone and in the peristellate zone gave the usual reactions for fibrinoid and sometimes stained metachromatically. Chromatropic substances also occurred between the fibers and in this site appeared to be more sensitive

to the action of hyaluronidase. In frozen sections the fibrillar structure was less apparent and there was a much more intense metachromatic reaction. The amount of metachromatic material lost on formalin fixation was considerable. In frozen sections, too, the metachromatic substance was found to extend somewhat into the necrotic zone and into the peristellate area. In association with the lesion there was also sclerosis of the blood vessels with hyperplasia, thickening, and hyalinization of the intima and media, and an increase of mucoid material in the adventitia. In certain of the blood vessels, aldehyde-positive thrombi were found. The nerves, at times, appeared edematous and contained an increased amount of hyaluronidase-sensitive metachromatic material (Figs. 5 and 6).

In early lesions of the synovial membrane, in which the cellular exudate was relatively small, there was an increase in metachromasia which was almost entirely absent when the section had undergone prior treatment with hyaluronidase. The acellular, homogeneous, refractile, deeply eosinophilic band-like structures in the underlying tissue retained some metachromasia under the same conditions. In this stage we observed some villosity of the synovial lining, and the villous projections, too, contained hyaluronidase-sensitive substances. In older lesions the cellular exudate and fine fibrillar precipitate increased and apparently progressed to the formation of hyaline-like masses in the sub-synovia which had all of the staining characteristics of fibrinoid. Deeper in the membranes the wide, finely fibrillar and relatively acellular bands might stain metachromatically with toluidine blue, give positive aldehyde and arginine reactions, stain red with van Gieson, and not stain deeply eosinophilically with hematoxylin and eosin. The alterations in vessels and nerves as described in the nodules also appeared in the synovial membrane. As in rheumatic fever, increased numbers of mast cells were observed in subcutaneous nodules and synovial membranes (Figs. 7 and 8).

Cysts, Bursae, and Ganglia

Fibrinoid formations were observed in cysts, bursae, and ganglia. In some instances this formation seemed to be related to overt necrosis, but in other cases no such relationship was apparent. In these sites, fibrinoid might be metachromatic with toluidine blue and give the red reaction with the van Gieson technic. In areas the orange or the yellow reaction in fibrinoid was apparent, however, and there the red and yellow fibers were continuous with each other. The fibrinoid substance was distinguished from the finely fibrillar, wide, relatively acellular material which was not intensely eosinophilic and was metachromatic, stained

red with the van Gieson, and reacted positively with the aldehyde reagent. This structure was identical histologically with that described in the deep layers of the sub-synovia in the cases of rheumatoid arthritis and at a distance in the peristellate zone of the rheumatoid nodule.

The blood vessels in these structures showed a variety of alterations. The changes ranged from edema of the walls to hyalinization and sclerosis. When the walls of the vessels were edematous or reticular in appearance, they contained an increased amount of hyaluronidase-insensitive metachromatic material and when hyalinized the metachromatic reaction might be absent but the aldehyde reaction was strongly positive. When thrombi were present, they might be metachromatic or normochromatic, but they were always aldehyde-positive. The nerves, too, showed comparable changes. The perineurium and endoneurium were thickened, and the nerves might contain increased quantities of a hyaluronidase-sensitive metachromatic substance. In the nerves there might be small hyaline bodies present which apparently arose from the small vessels. The contents of some of these structures have been described previously as containing hyaluronic acid.[28] In the sections studied, hyaluronidase-sensitive metachromatic material was noted as a constituent of the fluid in all three structures.

Disseminated Lupus Erythematosus

In the tissues of patients with disseminated lupus erythematosus metachromatic material accumulated in many sites in which fibrinoid characteristically occurs. Thus, chromatropic substances were demonstrated in affected skin, pockets and surfaces of valves, serosal surfaces, necrotic nodes, and glomeruli. The fibrinoid material in these various lesions reacted in the usual way with the technic utilized and demonstrated the same type of temporal, spatial, and configurational relationship to the metachromatic substances.

Klemperer[66] has noted a marked increase in chromatropic material in the hearts of some cases of disseminated lupus. This finding was corroborated. In the kidneys there was focal interstitial accumulation of metachromatic material in the cortices and marked metachromatic staining in the medullary substrate. In the cortex the accumulation seemed to be more marked perivascularly, especially at the vascular poles of the affected glomeruli and in the periglomerular area.

The chromatropic substances in the heart, skin, and serosal surfaces were sensitive to hyaluronidase in early lesions. As the lesions became older, it appeared that greater resistance to hyaluronidase developed.

In some sites, as in the glomeruli, the chromatropic substances usually were insensitive.

As in rheumatic fever, rheumatoid arthritis, and the other diseases studied, vascular and neural lesions (Fig. 9) occurred and the general pattern of alteration was the same.

Periarteritis Nodosa

In the acute necrotizing lesions of periarteritis nodosa the deeply eosinophilic material gave all of the staining reactions of fibrinoid. A blue fibrillar material, presumably fibrin, was observed in some of the phosphotungstic acid hematoxylin preparations. In serial sections it was noted that the blue material could not be correlated anatomically with the deeply eosinophilic substance. When the aldehyde reaction was utilized, the reaction was brilliantly red (Fig. 10) and the anatomic correlation with fibrinoid was good. Mast cells also were present in considerable numbers in sections of periarteritis nodosa.

Miscellaneous

In addition to the diseases studied above, the following structures also were investigated.

Gastric Ulcer. The toluidine blue reaction divided the area at the base of a chronic gastric ulcer roughly into three layers. The most superficial was deep blue, indicating a high concentration of nuclear material. This was confirmed by the Feulgen reaction. The middle layer did not stain with toluidine blue, and the lack of reaction has been taken to indicate the alkalinity of the layer. The arginine reaction was well marked in this area. The deeper layer stained metachromatically. Fibrinoid material began in the middle layer and extended for variable distances into the deeper layers. Again, fibrinoid material might or might not stain metachromatically with toluidine blue. With the aldehyde stain, with phosphotungstic acid hematoxylin, with van Gieson's stain, and with the arginine reagent, the staining reactions were characteristic for fibrinoid. Fibrin, when it occurred, might or might not be in the area of fibrinoid formation.

Placenta. The fibrinoid material in Nitabuch's membrane, that in the subchorial plate, and that involving the degenerative chorionic villi gave the same staining reactions as fibrinoid occurring elsewhere. Phosphotungstic acid hematoxylin stains indicated that fibrin might be precipitated in considerable quantities in some areas. It could not be correlated, however, with the distribution of the fibrinoid material.

DISCUSSION OF RESULTS

The distribution of fibrinoid suggests that the ground substance of the connective tissue is the only constant anatomic element in its formation. The occurrence of fibrinoid or equivalents in characteristic anatomic areas would seem to rule out collagen, reticulum, elastin, or muscle as invariable components. Fibrinoid-like changes may be produced by treating slices of umbilical cord with protamine, and fibrinoid-like structures may be observed in some cases of omphalitis or in the ground substance of the renal medulla. Some free bodies in the joint spaces are believed to be almost pure fibrinoid. Acellular, homogeneous, refractile eosinophilic bands are noted in the vitreous of some diseased eyes. In these specimens there is no organization of the "congealed" substance in question.

Other evidence points to the essential rôle of the amorphous ground substance. Fibrinoid which may not stain metachromatically with toluidine blue may do so in frozen sections or with some other basic aniline dye. In addition, there is a temporal, spatial, and configurational relationship between the metachromatic material and the fibrinoid substance. Talalajew's findings[23] with regard to the temporal relationship of the mucinous edema and fibrinoid alteration in rheumatic fever are substantiated by the observations in rheumatoid arthritis and in some of the other diseases studied. It has been observed that when the fibrinoid material is not metachromatic it is often in close proximity to the metachromatic substance and at times chromatropic substances actually appear to fuse with it. This fusion is well observed with the use of the van Gieson technic. The type of configurational relationship between the two substances is clearly illustrated in the Aschoff nodule.

In this investigation fibrinoid has invariably given a positive result with the Schiff reagent after periodic acid oxidation. The reaction with phosphotungstic acid hematoxylin has ruled out fibrin as an invariable component. Although the Feulgen reaction may be positive in or near the area of fibrinoid formation (in which instance the fibrinoid may or may not take on a basophilic hue), it is not invariably present. It may be considered that the liberation of desoxyribonucleic acid indicates the availability of a basic histone to react with the acid mucopolysaccharide. However, this need not always be the case, since pyknosis and karyorrhexis have been observed in the umbilical cord treated with protamine. If a basic protein is the precipitant, as would be suggested, it is quite conceivable that the necrosis of collagen, elastin, or muscle, or the liberation of a basic toxin, might give rise to this substance.

The Sakaguchi reaction [49,50] has indicated that fibrinoid contains considerable quantities of arginine. Some of the difficulties of the interpretation of this test have been mentioned. However, that this reaction may indicate the presence of a basic protein in these instances is supported by the reaction with toluidine blue (the basic dye is not attracted to the area of fibrinoid unless considerable quantities of nucleic acid or acid mucopolysaccharide are present), the increased eosinophilia, and the experimental work cited above.

The early increase in metachromatic material apparently is not specific for the fibrinoid change. It seems that the metabolism of the tissue is so altered in early lesions that the tissues are induced to produce increased quantities of acid mucopolysaccharides. An increase in chromatropic substance has been noted in tissues of patients with diabetes mellitus, myxedema, and in other conditions not generally associated with fibrinoid formation.

The significance to be ascribed to the mast cells is not clear. The distribution of the cells does not indicate that they are intimately involved in the formation of the metachromatic material found in the intercellular spaces.

This investigation has indicated that the formation of the metachromatic material is closely related to processes which have customarily been designated as hyalinization, amyloid formation, and sclerosis, as well as fibrinoid formation. The exact relationship and differences are not known.

The physiologic implications of alterations of this type must be profound, but in the present state of knowledge it seems premature to enter into a detailed discussion of this subject.

CONCLUSIONS

The mucinous edema of Talalajew [28] is associated with an increase in acid mucopolysaccharides.

The common feature of fibrinoid formation is the precipitation of the acid mucopolysaccharide of the ground substance of the connective tissue.

The precipitant in some instances is probably an alkaline protein derived from the necrosis of tissue or the interaction of the tissue with a damaging agent.

REFERENCES

1. Neumann, E. Die Picrocarminfärbung und ihre Anwendung auf die Entzündungslehre. *Arch. f. mikr. Anat.*, 1880, **18**, 130–150.

2. Ricker, G. Die Verflüssigung der Bindegewebsfasern. Zugleich ein Beitrag zur Kenntniss der fibrinoiden Degeneration. *Virchows Arch. f. path. Anat.*, 1901, **163**, 44–75.

3. Askanazy, M. Über Bau und Entstehung des chron. Magengeschwürs, sowie Soorpilzbefunde in ihm. *Virchows Arch. f. path. Anat.*, 1921, **234**, 111–178.

4. Klinge, F. Der Rheumatismus; pathologisch-anatomische und experimentell-pathologische Tatsachen und ihre Auswertung für das ärztliche Rheumaproblem. *Ergebn. d. allg. Path. u. path. Anat.*, 1933, **27**, 1–351.

5. Schosnig, F. Das Gewebsbild des fieberhaften Rheumatismus. Das Verhalten der Fasern des kollagenen Bindegewebes bei Rheumatismus und anderen Entzündungen. *Virchows Arch. f. path. Anat.*, 1932, **286**, 291–302.

6. Bennet, G. A., Zeller, J. W., and Bauer, W. Subcutaneous nodules of rheumatoid arthritis and rheumatic fever; a pathologic study. *Arch. Path.*, 1940, **30**, 70–89.

7. Gruber, G. B. Zur Frage der Periarteriitis nodosa, mit besonderer Berücksichtigung der Gallenblasen- und Nieren-Beteiligung. *Virchows Arch. f. path. Anat.*, 1925, **258**, 441–501.

8. Masugi, M., and Yä-Shu. Die diffuse Sklerodermie und ihre Gefässveränderung. *Virchows Arch. f. path. Anat.*, 1938, **302**, 39–62.

9. Pollack, A. D. Visceral and vascular lesions in scleroderma. *Arch. Path.*, 1940, **29**, 859–861.

10. Jäger, E. Zur pathologischen Anatomie der Thrombangiitis obliterans bei juveniler Extremitätengangrän. *Virchows Arch. f. path. Anat.*, 1932, **284**, 526–583; 584–622.

11. Klemperer, P., Pollack, A. D., and Baehr, G. Pathology of disseminated lupus erythematosus. *Arch. Path.*, 1941, **32**, 569–631.

12. Schürmann, P., and MacMahon, H. E. Die maligne Nephrosklerose, zugleich ein Beitrag zur Frage der Bedeutung der Blutgewebsschranke. *Virchows Arch. f. path. Anat.*, 1933, **291**, 47–218.

13. Jucker, P. Über die Nekrose in der arteriosklerotischen Platte und ihre Beziehung zum Atherom. *Virchows Arch. f. path. Anat.*, 1935, **295**, 301–333.

14. Grosser, O. Über Fibrin und Fibrinoid in der Placenta. *Ztschr. f. Anat. u. Entwcklngsgesch.*, 1925, **76**, 304–314.

15. Wu, T. T. Über Fibrinoidbildung der Haut nach unspezifischer Gewebsschädigung bei der Ratte. *Virchows Arch. f. path. Anat.*, 1937, **300**, 373–377.

16. Meessen, H. Über Coronarinsuffizienz nach Histamincollaps und nach orthostatischem Collaps. *Beitr. z. path. Anat. u. z. allg. Path.*, 1937, **99**, 329–350.

17. Henschen, F. Über eine eigenartige, mit Thrombenbildung verbundene Reaktion des Gefässendothels. *Acta med. Scandinav.*, 1926–27, **65**, 539–554.

18. Selye, H. The general adaptation syndrome and the diseases of adaptation. *J. Clin. Endocrinol.*, 1946, **6**, 117–230.

19. Gerlach, W. Studien über hyperergische Entzündung. *Virchows Arch. f. path. Anat.*, 1923–24, **247**, 294–361.

20. Clark, E., Graef, I., and Chasis, H. Thrombosis of the aorta and coronary arteries; with special reference to the "fibrinoid" lesions. *Arch. Path.*, 1936, **22**, 183–212.

21. Bahrmann, E. Über die fibrinoide Degeneration des Bindegewebes. *Virchows Arch. f. path. Anat.*, 1937, **300**, 342–372.

22. Klemperer, P., Pollack, A. D., and Baehr, G. Diffuse collagen disease. Acute disseminated lupus erythematosus and diffuse scleroderma. *J. A. M. A.*, 1942, **119**, 331–332.

23. Talalajew, W. T. Der akute Rheumatismus. *Klin. Wchnschr.*, 1929, **8**, 124–129.

24. Geipel, P. Untersuchungen über rheumatische Myokarditis. *Deutsches Arch. f. klin. Med.*, 1905–06, **85**, 75–88.

25. Baehr, G., and Pollack, A. D. Disseminated lupus erythematosus and diffuse scleroderma. *J. A. M. A.*, 1947, **134**, 1169–1174.

26. Klemperer, P. Diseases of the collagen system. *Bull. New York Acad. Med.*, 1947, **23**, 581–588.

27. Duran-Reynals, F. Tissue permeability and the spreading factors in infection; a contribution to the host:parasite problem. *Bact. Rev.*, 1942, **6**, 197–252.

28. Meyer, K. The biological significance of hyaluronic acid and hyaluronidase. *Physiol. Rev.*, 1947, **27**, 335–359.

29. Clark, E. R., and Clark, E. L. Further observations on living lymphatic vessels in the transparent chamber in the rabbit's ear. Their relation to the tissue spaces. *Am. J. Anat.*, 1933, **52**, 273–305.

30. Bensley, S. H. On the presence, properties and distribution of the intercellular ground substance of loose connective tissue. *Anat. Rec.*, 1934, **60**, 93–109.

31. Chain, E., and Duthie, E. S. Identity of hyaluronidase and spreading factor. *Brit. J. Exper. Path.*, 1940, **21**, 324–338.

32. McClean, D. The influence of testicular extract on dermal permeability and the response to vaccine virus. *J. Path. & Bact.*, 1930, **33**, 1045–1070.

33. Meyer, K. Mucoids and Glycoproteins. In: Anson, M. L., and Edsall, J. T. (eds.) Advances in Protein Chemistry. Academic Press, Inc., New York, 1945, **2**, 249–275.

34. Stacey, M. The Chemistry of Mucopolysaccharides and Mucoproteins. In: Pigman, W. W., and Wolfrom, M. L. (eds.) Advances in Carbohydrate Chemistry. Academic Press, Inc., New York, 1946, **2**, 161–201.

35. Meyer, K. The chemistry and biology of mucopolysaccharides and glycoproteins. *Cold Spring Harbor Symposia on Quantitative Biology*, 1938, **6**, 91–102.

36. Schade, H. Die physikalische Chemie in der inneren Medizin. T. Steinkopff, Dresden & Leipzig, 1923, 605 pp.

37. Knepper, R. Die Ionenkonzentration der allergisch-hyperergischen Entzündung. *Klin. Wchnschr.*, 1937, **16**, 188–190.

38. Schade, H. Die Methodik der H-Ionenmessung an offenen Wundflächen und ihre Ergebnisse. *München. med. Wchnschr.*, 1926, **73**, 343–344.

39. Koller, F., and Leuthardt, F. Nekrose und Autolyse. Beitrag zur Kenntnis der dystrophischen Verkalkung. *Klin. Wchnschr.*, 1934, **13**, 1527–1529.

40. Wiechmann, E., and Paal, H. Zur Genese des Asthma bronchiale. *Klin. Wchnschr.*, 1925, **4**, 823–824.

41. Ellinger, P. Pathologische Physiologie und Pharmakotherapie des Asthma bronchiale. *Klin. Wchnschr.*, 1931, **10**, 1105–1109.

42. Tiefensee, K. W. Probleme der Asthmaforschung. *München. med. Wchnschr.*, 1933, **80**, 309–312.

43. Holmgren, H. Eine neue Methode zur Fixierung der Ehrlichschen Mastzellen. Mit besonderer Berücksichtigung der Chemie der Zellgranula. *Ztschr. f. wissensch. Mikr.*, 1938, **55**, 419–461.

44. Mullen, J. P., and McCarter, J. C. A mordant preparing formaldehyde-fixed neuraxis tissue for phosphotungstic acid hematoxylin staining. *Am. J. Path.*, 1941, **17**, 289–291.

45. McManus, J. F. A. Histological demonstration of mucin after periodic acid. *Nature, London*, 1946, **158**, 202.

46. Hotchkiss, R. D. A microchemical reaction resulting in the staining of poly-saccharide structures in fixed tissue preparations. *Arch. Biochem.*, 1948, **16**, 131–141.

47. Feulgen, R., and Voit, K. Über einen weitverbreiteten festen Aldehyd. *Arch. f. d. ges. Physiol.*, 1924, **206**, 389–410.

48. Stowell, R. E. Feulgen reaction for thymonucleic acid. *Stain Technol.*, 1945, **20**, 45–58.

49. Weber, C. J. A modification of Sakaguchi's reaction for the quantitative determination of arginine. *J. Biol. Chem.*, 1930, **86**, 217–222.

50. Serra, J. A. Histochemical tests for proteins and amino acids; the characterization of basic proteins. *Stain Technol.*, 1946, **21**, 5–18.

51. Jackson, E. L., and Hudson, C. S. The structure of the products of the periodic acid oxidation of starch and cellulose. *J. Am. Chem. Soc.*, 1938, **60**, 989–991.

52. Lison, L. Histochimie animale. Méthodes et problèmes. Gauthier-Villars, Paris, 1936, 320 pp.

53. Kelley, E. G., and Miller, E. G., Jr. Reactions of dyes with cell substances. I. Staining of isolated nuclear substances. *J. Biol. Chem.*, 1935, **110**, 113–118.

54. Wislocki, G. B., Bunting, H., and Dempsey, E. W. Metachromasia in mammalian tissues and its relationship to mucopolysaccharides. *Am. J. Anat.*, 1947, **81**, 1–37.

55. Dempsey, E. W., Bunting, H., Singer, M., and Wislocki, G. B. The dye-binding capacity and other chemo-histological properties of mammalian mucopolysaccharides. *Anat. Rec.*, 1947, **98**, 417–429.

56. Wiame, J. M. The metachromatic reaction of hexametaphosphate. *J. Am. Chem. Soc.*, 1947, **69**, 3146–3147.

57. Unpublished experiments.

58. Michaelis, L., and Granick, S. Metachromasy of basic dyestuffs. *J. Am. Chem. Soc.*, 1945, **67**, 1212–1219.

59. Block, R. J., and Bolling, D. The Amino Acid Composition of Proteins and Foods. Charles C Thomas, Springfield, Illinois, 1947, 396 pp.

60. Gross, L., and Ehrlich, J. C. Studies on the myocardial Aschoff body. I. Descriptive classification of lesions. *Am. J. Path.*, 1934, **10**, 467–487.

61. Karsner, H. T., and Bayless, F. Coronary arteries in rheumatic fever. *Am. Heart J.*, 1933–34, **9**, 557–585.

62. Fröhlich, A. Ueber lokale gewebliche Anaphylaxie. *Ztschr. f. Immunitätsforsch. u. exper. Therap.*, 1913–14, **20**, 476–500.

63. Lasowsky, J. M., and Kogan, M. M. Die Beteiligung des Nervensystems an allergischen Prozessen. Morphologische Veränderungen der Nervenfasern bei norm- und hyperergischer Entzündung der Skeletmuskulatur. *Virchows Arch. f. path. Anat.*, 1934, **292**, 428–441.

64. Schultz, A. Über einen Fall von Athyreosis congenita (Myxödem) mit besonderer Berücksichtigung der dabei beobachteten Muskelveränderungen. *Virchows Arch. f. path. Anat.*, 1921, **232**, 302–315.

65. Krücke, W. Die mucoide Degeneration der peripheren Nerven. *Virchows Arch. f. path. Anat.*, 1939, **304**, 442–463.

66. Klemperer, P. The pathogenesis of lupus erythematosus and allied conditions. *Ann. Int. Med.*, 1948, **28**, 1–11.

[*Illustrations follow*]

DESCRIPTION OF PLATE

PLATE 172

Fig. 1. Section of the mitral valve from a patient with acute rheumatic fever, stained with toluidine blue. The metachromatic material is concentrated particularly beneath the proliferated endothelium. × 70.

Fig. 2. Same tissue as shown in Figure 1, treated with hyaluronidase prior to staining. × 70.

Fig. 3. Myocardium from a patient with acute rheumatic fever. The oval or spindle-shaped metachromatic nodules with cellular aggregates are Aschoff bodies. × 70.

Fig. 4. An Aschoff body stained with leukofuchsin after periodic acid oxidation. × 200.

Fig. 5. Section of a subcutaneous nodule from a patient with rheumatoid arthritis, stained with toluidine blue. × 70.

Fig. 6. Similar to Figure 5. Stained with phosphotungstic acid hematoxylin. No fibrin is apparent in this section. × 85.

Fig. 7. Section of a synovial membrane from a patient with rheumatoid arthritis. Stained with toluidine blue. × 150.

Fig. 8. Same tissue as shown in Figure 7, treated with hyaluronidase prior to staining. × 150.

Fig. 9. Peripheral nerve from a patient with disseminated lupus erythematosus. The metachromatic material is sensitive to hyaluronidase. The shrinkage of tissue is due to the vigorous dehydration technic necessary to maintain metachromasia. × 85.

Fig. 10. Section from the gallbladder wall of a patient with periarteritis nodosa, stained with leukofuchsin after periodic acid oxidation. × 85.

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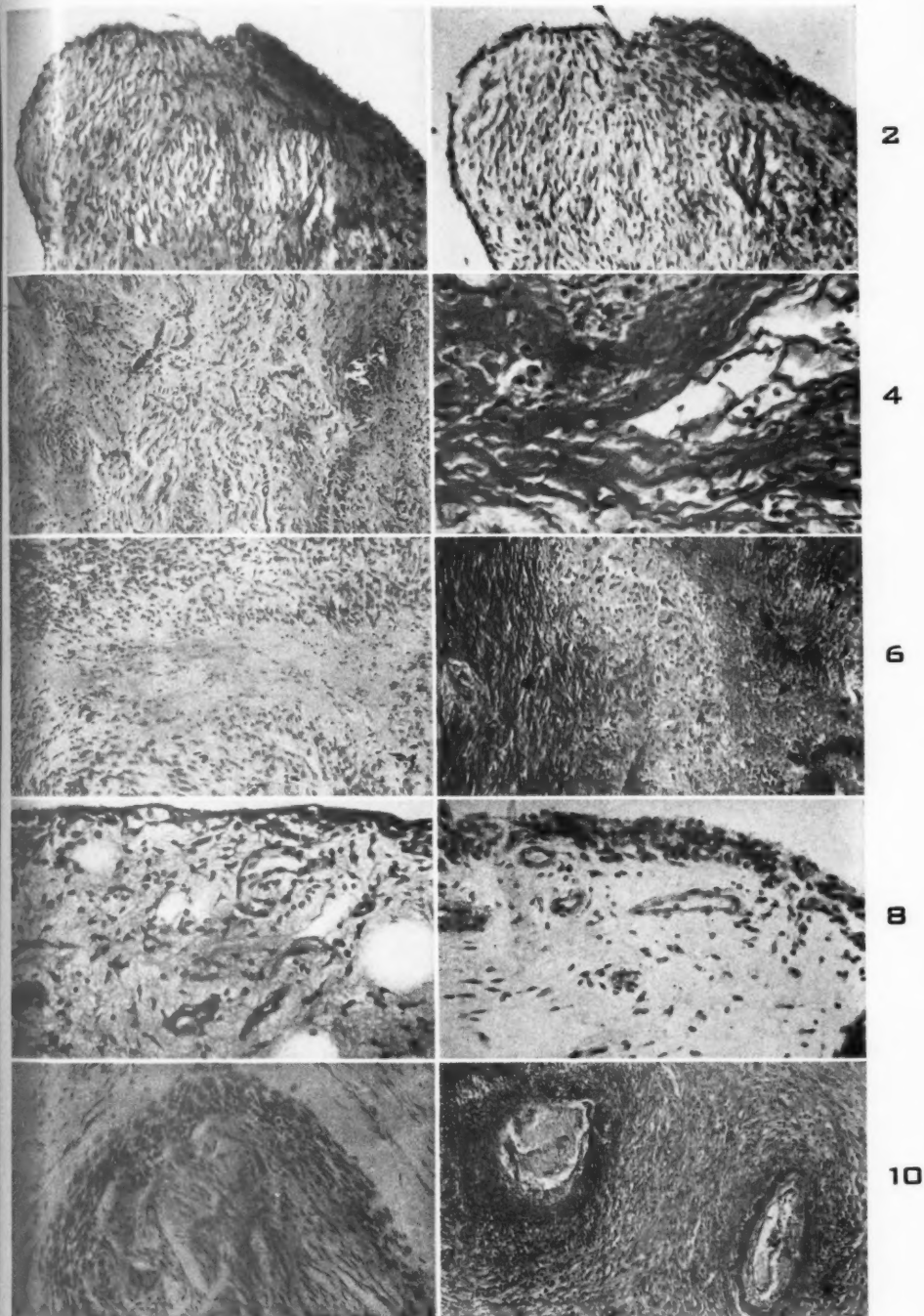
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MICROCHEMICAL VARIATION OF ALKALINE PHOSPHATASE ACTIVITY OF LIVER IN OBSTRUCTIVE AND HEPATOCELLULAR JAUNDICE*

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The importance of the liver in excreting alkaline phosphatase into the bile has been well established in experimental biliary obstruction,[1,2] after hepatectomy in dogs,[3-5] and in the clinical determination of serum levels in disease of the biliary tract.[6-8] The precise mechanism of excretion by the liver is unknown. A fundamental difference apparently exists between the mode of excretion of bilirubin and of alkaline phosphatase. In total or partial biliary obstruction the two substances are retained in the blood stream. However, in jaundice due to disease of the liver cells, little or no retention of alkaline phosphatase may occur.[9]

Microchemical studies of the concentration and localization of alkaline phosphatase in normal and diseased livers of man, and in normal and experimentally obstructed or diseased livers of animals[10-16] have provided information which may be related to the excretory activities of hepatic lobular structures. The study reported here is a further attempt to correlate the variation in hepatic alkaline phosphatase determined microchemically with changes of phosphatase in the blood stream in experimental animal and human obstructive and hepatocellular jaundice.

METHODS

Histochemical. Alkaline phosphatase activity in tissue sections was demonstrated by the method of Gomori and Takamatsu.[11,17] Tissue sections were incubated in a solution of calcium nitrate and sodium glycerophosphate at pH 9.1. At the sites of phosphatase activity PO_4 ions were split from the ester and calcium phosphate precipitates. These colorless salts were transformed to brown silver phosphate by exposure of the sections in silver nitrate to ultraviolet light. Sections were incubated in the substrate mixture at 38° C. for 2 hours in every case. Time intervals and temperatures for embedding, and the period of exposure of the sections in silver nitrate solution to ultraviolet light at uniform distance and intensity were standardized in order to obtain results which were reproducible in consecutive serial sections. Absolute alcohol was used for fixation. Experience with liver had shown diffusion of enzyme activity in the central portions of sections from blocks of ordinary size, due presumably to post-mortem diffusion of the enzyme during the

period of penetration of the fixative. Localization of the enzyme activity was often strikingly more precise at the periphery of the sections where penetration of the fixative was most rapid. To achieve a minimum of diffusion, liver tissue was cut in blocks of 1 to 2 mm. in each dimension.

Chemical. The method of King and Armstrong[18] was used to determine the alkaline phosphatase activity of duplicate extracts of each of two widely separated areas of liver tissue in the animal experiments. Serum alkaline phosphatase activity was determined by the method of Bodansky[19] in human subjects, and by the method of King and Armstrong[18] in the animals.

Experimental. The effect of total extrahepatic biliary obstruction on the distribution of alkaline phosphatase in the liver was studied in dogs and cats. Under nembutal anesthesia and with the usual aseptic precautions, liver tissue was obtained for histochemical and chemical studies. The common bile duct was then doubly ligated and cut, and the gallbladder removed. Blood for the determination of serum alkaline phosphatase and bilirubin was obtained prior to operation and at intervals thereafter to the end of the experiment. On the seventh to ninth postoperative day the animals were killed and liver was obtained for histochemical and chemical examination.

The intrahepatic distribution of alkaline phosphatase in hepatic necrosis produced by chloroform or by carbon tetrachloride was studied in dogs. The dogs were allowed to recuperate for 1 week after specimens of liver were obtained for controls. After all food was withdrawn for 24 hours, each dog was given either 10 to 20 cc. of chloroform intratracheally or 1.0 to 2.0 cc. of carbon tetrachloride per kg. of body weight, mixed with an equal quantity of ethyl alcohol, by stomach tube. Two to 7 days later the animals were killed and liver specimens obtained. Serum alkaline phosphatase and bilirubin determinations were made before and after the drugs were given.

RESULTS

Normal Adult Human Liver Tissue

Table I summarizes semiquantitatively, the histochemical distribution of alkaline phosphatase in 13 adult human livers which were normal in sections stained with hematoxylin and eosin. While there was some individual variation, the general amount and localization were much the same in all, and the findings were fairly uniform throughout individual livers (Fig. 1). Activity was predominantly in the Kupffer cells and other endothelial cells of the hepatic sinuses, usually being present in all but with slightly greater activity in the sinus lining cells of the periportal

TABLE I

TABLE I

Distribution of Alkaline Phosphatase in Normal Adult Human Liver*

Case no.	Sex	Age	Main diagnosis at autopsy	Bile canaliculi	"Ruptured" bile canaliculi†	Sinusoidal cells			Hepatic cell cytoplasm	Bile duct lumina
						Periportal area	Mid-zone	Central area		
1	M	62	Encephalomalacia	o	o	++	o	o	o	+
2	F	45	Subarachnoid hemorrhage	++	o	+++	+++	+	o	o
3	M	28	Polycystic kidneys	++	o	++	+++	+	o	o
4	M	62	Carcinoma, bronchus	o	o	+++	+++	+	o	o
5	M	52	Subacute bacterial endocarditis	++	o	+++	+++	+	o	o
6	M	40	Lobar pneumonia	+	++	+++	+++	++	o	++
7	M	42	Burns	o	o	+++	+++	++	o	o
8	F	56	Carcinoma, cervix	+	o	+++	+++	++	o	o
9	M	30	Brain abscess	++	o	+++	++	++	o	o
10	F	39	Subacute bacterial endocarditis	++	o	+++	+	+++	o	++
11	F	42	Carcinoma, cervix	+	++	+++	++	++	o	+
12	F	24	Acute bacterial endocarditis	o	o	+	++	++	o	o
13	M	73	Tuberculosis and silicosis	+	++	++	++	++	o	+

* Scale from o to ++++.
† Branches of the bile canaliculi, as outlined by the results of enzyme activity, occasionally extended to the space of Disse, thus apparently separating individual liver cells at the sinusoidal barrier where, in ordinary sections, they appeared to be contiguous.

and central zones. The cytoplasm of the liver cells was strikingly devoid of enzyme activity. Traces were found in the hepatic cell nuclei. Reticulum lining the sinuses was encrusted with granules, indicating enzyme activity on or in these fibrils. In most normal livers the results of activity in the bile canaliculi delineated these structures as thin branching lines between the liver cells and extending toward the sinuses. Occasionally in normal liver these branch canaliculi, outlined by granules indicating enzyme activity, extended to the space of Disse. This was a common finding in biliary obstruction, and will be described below. Slightly more activity was noted in the canaliculi of the central and periportal zones, as was seen in the cells lining the sinuses. A slight amount of activity was found inconsistently in the lumina of the bile ducts, in the endothelium of efferent veins, and in the endothelium of capillaries near bile ducts.

TABLE II

Distribution of Alkaline Phosphatase

Case	Sex	Age	Main diagnosis	Duration of jaundice	Serum determinations			
					Bilirubin (mg. %)	Alkaline phosphatase (Bodansky units %)	Total cholesterol (mg. %)	Cephalin flocculation
1	M	58	Carcinoma, head of pancreas; secondary carcinoma in liver*	4 wks.	15.6 (4)‡	15.0 (4)‡	410 (31)‡	+
2	F	49	Postoperative stricture of common duct; biliary fistula*	1 yr.	16.7 (3)	26.2 (3)	303 (3)	Neg.
3	F	49	Postoperative stricture of common duct*	4 yrs. intermit.	4.0 (10)	53.6 (10)	390 (10)	+ (10)‡
4	M	68	Carcinoma of common duct with liver metastases*	3 wks.	28.8 (4)	22.9 (9)	585 (8)	Neg. (8)
5	M	48	Large multilocular liver abscess†	Terminal	4.8 (0)	17.3 (0)		3+
6	M	57	Carcinoma of stomach, invading and obstructing common duct; liver metastases†	6 wks.	9.4 (28)	14.4 (28)	222 (28)	Neg. (28)
7	F	61	Carcinoma of hepatic ducts; liver metastases; extension into portal veins†	5 mos.	25.0 (21)	26.8 (21)	436 (21)	Neg. (21)
8	F	1¼	Congenital atresia of bile ducts†	1 yr.	6.7 (8 mos.)	41.0 (8 mos.)	156 (8 mos.)	+ (8 mos.)
9	M	82	Carcinoma of hepatic ducts†	6 wks.	27.3 (4)	22.9 (4)	276 (4)	+ (4)
10	M	30	Carcinoma of papilla of Vater; Cholecystoduodenostomy†	2 yrs., intermit.	15.9 (2)	14.4 (2)	195 (2)	Neg. (2)
11	F	64	Common duct calculi; choledochostomy 15 hours before death†	1 mo., intermit.	3.8 (1)	7.4 (6)		Neg. (6)
12	M	75	Common duct calculi; biliary fistula†	3 wks.	7.7			
13	M	59	Small carcinoma of papilla of Vater; "T" tube in common duct 8 days before death; cholecystojejunostomy†	7 wks.	22.0 (14)	21.7 (14)	750 (14)	

* Biopsy.

† Post mortem.

‡ Figure in parentheses is the number of days before biopsy or autopsy was done.

Human Liver Tissue in Obstructive Jaundice

The findings in 13 cases of jaundice due to partial or complete obstruction of the extrahepatic or intrahepatic bile ducts are summarized in Table II. The enzyme activity was found in the sinusoidal cells, the

in Human Liver with Obstructive Jaundice

Histologic examination of liver tissue				
	Alkaline phosphatase			
Hematoxylin and eosin stain	Bile canaliculi	Sinusoidal cells	Liver cells	"Rupture" of bile canaliculi
Obstructive jaundice, bile plugs in canaliculi, early biliary cirrhosis	4+	4+	o	4+
Biliary cirrhosis, pericholangitis, bile plugs in canaliculi	4+	4+	o	3+
Biliary cirrhosis, pericholangitis, bile plugs in canaliculi	3+	3+	o	3+
Obstructive jaundice, bile plugs in canaliculi	3+	3+	o	3+
Focal and central necrosis of liver cells, no bile plugs in canaliculi	3+	3+	+	2+
Bile plugs in canaliculi, biliary cirrhosis	3+	2+	o	+
Severe central necrosis of liver cells, bile plugs in canaliculi and Kupffer cells, early biliary cirrhosis	+	+	+	o
Advanced biliary cirrhosis, fatty liver, many bile plugs	2+	3+	o	+
Biliary cirrhosis, bile plugs in canaliculi, focal liver cell necrosis	4+	4+	+	4+
Biliary cirrhosis, pericholangitis, bile plugs in canaliculi, focal necrosis of liver cells	2+	3+	o	+
Pericholangitis, early biliary cirrhosis	+	2+	o	o
Slight passive congestion	+	2+	o	+
Early biliary cirrhosis, bile pigment in canaliculi of central zones	+	2+	o	+

sinus reticulum, and in the bile canaliculi as in the normal liver but the quantity of these locations was very greatly increased (Fig. 2). The distribution was uniform in contrast to the more central preponderance of bile pigment in obstruction and to the central and periportal predominance of enzyme activity in the normal liver. Dilated bile canaliculi and their small branches between liver cells were filled uniformly with masses

of bile pigment. Regurgitation of bile between liver cells to the space of Disse was plainly demonstrated by the appearance of branch canaliculi as outlined by the results of enzyme activity. Most of the branch canaliculi completely separated individual liver cells and effected a junction with the space of Disse.

There is an approximate correlation between the amount of enzyme activity in the serum and that demonstrable histochemically in the bile canaliculi and in the sinusoidal lining cells. In cases of carcinoma obstructing the bile ducts or of stricture (cases 1, 2, 3, 4, 6, and 9), high serum levels of enzyme activity were reflected in the liver by greatly increased activity in the canaliculi and sinusoidal cells. On the other hand, in cases of obstruction of the common duct by stone or tumor (cases 11, 12, and 13) in which bile stasis was relieved surgically and in which the serum enzyme activity was normal or only slightly elevated, the enzyme activity in the liver was normal or slightly increased. An exception to this correlation was case 7 in which the activity in the liver was much less than was expected from the serum level. It is possible that interference with hepatic blood flow by extensive invasion of the portal veins by tumor in this liver may have interfered with delivery of the enzyme to the liver lobules.

In obstructive jaundice as in the normal liver, the cytoplasm of the liver cells was devoid of alkaline phosphatase activity so far as could be demonstrated by the histochemical technic. Only in 3 cases in occasional liver cells undergoing necrosis was intracytoplasmic enzyme activity seen. Enzyme activity occasionally found limited to portions of the cytoplasm in juxtaposition to markedly active bile canaliculi and sinusoidal endothelial cells was attributed to diffusion. In some sections enzyme diffused over the surface of the sections during incubation, resulting in the precipitation of granules which appeared at first to be in the cytoplasm of the liver cells. Under high-power magnification the granules were found to be above the level of the tissue section. As in normal liver, hepatic cell nuclei showed traces of activity but there was no increase in the obstructed liver as compared with the normal.

Endothelial cells of the portal and efferent veins revealed moderately increased enzyme activity in obstruction. No granules were seen in the bile duct epithelium, but coagulum present in the bile duct lumina showed a small degree of activity. There was no activity in the portal connective tissue.

Normal Liver Tissue of the Dog

Table III summarizes the histochemical findings in 9 normal dog livers. Greater activity was seen in these sections as compared with

human liver (Fig. 3). The activity in the bile canaliculi accounted for the difference, the enzyme in the cells lining the sinuses being relatively less conspicuous. The concentration of enzyme in these locations is greater in the periportal zones. No activity was demonstrable in the cytoplasm of the liver cells but, as in man, the nuclei often showed traces of activity. Bile duct epithelium and bile duct lumina showed greater activity than in the same locations in man.

Liver Tissue of the Dog in Experimentally Produced Obstructive Jaundice

Armstrong, King, and Harris,[1] and Bodansky and Jaffe[2] were the first to show that the high serum alkaline phosphatase values encountered clinically in obstructive jaundice could be reproduced in dogs by ligation of the common bile duct. In similar experiments on 3 dogs (Fig. 4, Tables III and IV) to determine the distribution of enzyme and alteration of activity cytologically, the serum alkaline phosphatase activity increased

TABLE III

Distribution of Alkaline Phosphatase (Gomori-Takamatsu Histochemical Technic[11,17]) in Liver Tissue of Normal Dogs and Cats, and after Common Duct Ligation and Cholecystectomy

Animal no.	Relation of material to experimental procedure	Bile canaliculi	"Ruptured" bile canaliculi	Sinusoidal cells	Hepatic cells	Bile duct epithelium
Dog 352	Normal, biopsy	++	+	±	o	++
	9 days after ligation	+++	+++	++	o	+
Dog 353	Normal, biopsy	+	o	±	o	++
	9 days after ligation	+++	++	+	o	++
Dog 389	Normal, biopsy	+	o	+	o	+
	9 days after ligation	++++	+++	++	±	++
Dog 297	Normal, biopsy	++	+	+	o	++
Dog 260	Normal, biopsy	+	+	±	±	+
Dog 376	Normal, biopsy	o	o	+	o	+
Dog 898	Normal, biopsy	++	±	+	o	+
Dog 896	Normal, biopsy	++	o	+	o	+
Dog 881	Normal, biopsy	±	o	o	o	o
Cat 354	Normal, biopsy	o	o	+	o	o
	9 days after ligation	+++	++	++	o	±
Cat 394	Normal, biopsy	±	+	±	o	±
	9 days after ligation	++	+++	++	o	±

within a few days from 40 to 100 times the preoperative level. Extracts of the obstructed liver showed 1560 to 1800 King-Armstrong units of phosphatase activity per 100 gm. of wet tissue—2 to 4 times the activity found in extracts of the control specimens. Microchemically, the increase in activity, especially in the bile canaliculi, was found to be more marked than in obstruction in man. The greater activity was in the canaliculi of

TABLE IV

Chemical Analyses of Blood and Liver Tissue of Dogs and Cats before and after Common Duct Ligation and Cholecystectomy

Animal no., and weight		Bilirubin	Alkaline phosphatase	Average liver tissue alkaline phosphatase†	Remarks
		mg. %	K-A units %*		
Dog 352 16 kg.	Before ligation			810	
	2 days postop.		520.0		Tarry stools
	5 days postop.	2.0	575.0		Urine bile-stained
	9 days postop.	3.5	500.0	1250	Clay-colored stools Urine bile-stained
Dog 353 13.5 kg.	Before ligation		13.2	480	
	2 days postop.	2.2	595.0		Stools gray
	4 days postop.		450.0		Jaundiced
	7 days postop.	4.6	415.0		Jaundiced
	9 days postop.	3.9	525.0	1550	Jaundiced
Dog 389 8.5 kg.	Before ligation		9.0	450	
	2 days postop.	0.1	147.0		
	7 days postop.	0.2	550.0		No jaundice
	9 days postop.	0.3	550.0	1625	Stools clay-colored Urine dark
Cat 354	Before ligation		4.0	150	
	3 days postop.		7.0		Jaundiced
	5 days postop.	1.9	7.2		Jaundiced
	7 days postop.		4.7		Jaundiced
	9 days postop.	1.8	4.0	225	Jaundiced
Cat 394 4.5 kg.	Before ligation		10.5	350	
	2 days postop.		7.3		Jaundiced
	4 days postop.	6.6	36.0		Jaundiced
	6 days postop.		19.0		Jaundiced
	8 days postop.	7.8	36.0	650	Jaundiced

* King-Armstrong units per 100 gm. of wet tissue.
† Average of duplicate determinations of two widely separated tissue samples.

the periportal zones. The enzyme was more active in the endothelial cells of the sinuses but the increase in activity was less conspicuous than was found in man. The cytoplasm of liver cells showed no activity. However, occasional necrotic liver cells in the periportal zones showed activity in the cytoplasm. No increase in activity was noted in bile duct epithelium as compared with the specimens for biopsy. After obstruction, activity in endothelial cells lining portal and efferent veins increased.

Liver Tissue of Dogs with Mild and Severe Central Necrosis Due to Chloroform or Carbon Tetrachloride

Mild central lobular necrosis was produced in 4 dogs by intratracheal injection of chloroform (Table V). None became jaundiced but in all there was a transient elevation in serum alkaline phosphatase activity, which was slight in contrast to the increase noted in common duct obstruction. At autopsy the enzyme activity of bile canaliculi, bile duct epithelium, and sinusoidal lining cells was increased in comparison

with that of tissue taken before liver cell injury. The increase in activity of the canaliculi and endothelial cells was greatest in the central zones—the area of hepatic cell injury. In 2 of the dogs autopsied in the early phase of liver damage, necrotic cells of the central zones showed enzyme activity in the cytoplasm. In contrast with the dogs in which more severe damage was produced, there was no regurgitation of enzyme between liver cells.

The effect of complete destruction of liver cells on enzyme activity within the liver lobule was studied in 3 dogs to which carbon tetrachloride was given with ethyl alcohol by stomach tube. Bodansky[20] and Freeman, Chen, and Ivy[5] found that following administration of carbon tetrachloride to dogs the serum alkaline phosphatase level rose, but to only a fraction of the extraordinary levels observed after ligation of the common duct. Our own experiments using comparable dosages gave similar results (Table V), although they were interrupted sooner to obtain sections during the period of maximal damage. At autopsy there was complete disappearance of the liver cells in the central zones where the stroma was collapsed and the areas infiltrated by phagocytes. The liver cells in the remaining portion of the lobule were normal. In the damaged central zones no bile pigment or enzyme activity was seen. Cytoplasm of necrotic liver cells at the periphery of the damaged zone showed activity, but no activity was seen in the well preserved liver cells in the midportal and periportal zones. The phosphatase activity of bile canaliculi and sinusoidal lining cells of the undamaged zones was increased as compared with these zones in sections obtained for biopsy before poisoning (Fig. 5). In 2 dogs the microchemical appearance of the normal zones was similar to that of obstruction with the exception of the absence of bile pigment in the canaliculi. Most of the branch canaliculi had "ruptured" into the space of Disse.

Normal Liver and Kidney Tissue of the Cat

By both chemical and histochemical technics (Tables III and IV), normal cat liver was found to show less alkaline phosphatase activity than was found in that of the normal dog or in human liver. This difference is probably related to the fact that in the cat, the kidney plays an important rôle in the excretion of the enzyme. Cantarow, Stewart, and McCool[21] found that common duct ligation in cats is not followed by the very marked rise in serum alkaline phosphatase activity observed in man and dog under similar conditions. Flood, Gutman, and Gutman[22] showed that the cat excretes more enzyme in the urine than does man or

TABLE
Alkaline Phosphatase in Liver Necrosis of D... duced by

| Dog no. | Drug | Serum alkaline phosphatase (K-A units/100 cc.) | | Bilirubin (mg./100 cc.) | Day autopsied after drug | Liver lesion | Chemical K-A units/100 gm. of wet tissue | | Contr | |
		Peak level and day	At autopsy				Control biopsy	Autopsy		Liver cells
881	10 cc. CHCl₃ intratracheal	25.8 (2)*		1.1 (2)*	3	Moderate central necrosis	550	410	+	0
898	10 cc. CHCl₃	19.5 (3)	16.0	0 (2)	4	Mild central necrosis; fatty accumulation	630	2200	+	0
376	12 cc. CHCl₃; 25 cc. 5th day	19.0 (8)	13.1	0.2 (9)	9	Slight central necrosis; fatty accumulation; central regeneration	935	2280		0
861	15 cc. CHCl₃	14.8 (3)	3.8	0	9	Regeneration of central liver cells		275		
896	1.5 cc. CCl₄; 1.5 cc. C₂H₅OH	79.0 (3)	79.0	0.1 (2)	3	Severe central necrosis	255	1075	+	0
260	2 cc. CCl₄; 2 cc. C₂H₅OH	54.0 (6)	30.1	0.5 (3)	7	Patchy, severe central necrosis; central fatty accumulation; normal areas	400	230	+	0
297	15 cc. CCl₄; 15 cc. CCl₄	170.0 (5)	80.0	1.2 (9)	8	Severe central liver necrosis	320	520	+	0

* Figure in parentheses is the number of days before death when determination was made.

dog. The cat shows a remarkable species difference in that the renal glomeruli, unlike those of man and dog (Figs. 6 and 7), reveal great enzyme activity. In the normal cat liver the enzyme activity present, chiefly in the central portion of the lobules, was distributed in the sinusoidal lining cells and bile canaliculi in much the same way as in man and dog, none being found in the cytoplasm of liver cells.

Liver and Kidney Tissue of the Cat in Experimentally Produced Obstructive Jaundice

In 2 cats subjected to ligation of the common duct and cholecystectomy (Table IV), the serum phosphatase activity rose to only two to three times the preoperative level, as had been found by Cantarow.[23] Liver enzyme activity, as determined chemically, was increased less after obstruction than in the obstructed dogs (Table IV). Histochemically, there was less increase in enzyme in the bile canaliculi and fewer "ruptures" of canaliculi between liver cells as compared with the liver of the completely obstructed dog or man. This difference was not so great in respect to the activity of the sinusoidal lining cells. As in man and

TABLE

...is of De...duced by *Chloroform and Carbon Tetrachloride*

		Histochemical determination of alkaline phosphatase activity											
Chemical K-A 100 gm. of tissue		Control biopsy			Autopsy								
					Central zones			Mid- and periportal zones			"Rupture" of bile canaliculi	Bile duct lumina
Autopsy		Liver cells	Sinusoidal cells	Bile duct lumina	Bile canaliculi	Liver cells	Sinusoidal cells	Bile canaliculi	Liver cells	Sinusoidal cells		
410	+	o	++	o	++	++	++	++	o	++	o	++
2200	+	o	+	±	+++	±	+++	++	o	++	+	++
2280	o	o	+	+	+++	o	++	++	o	+	±	++
275					++++	o	+++	++	o	++	±	++
1075	+	o	+	+	o	o	o	++	o	++	+++	+++
230	+	o	±	+	o	o	o	+++	o	+++	±	++++
520	+	o	+	+	o	o	o	++++	o	+++	+++	+++

dog, the cytoplasm of the liver cells did not show activity after obstruction. A peculiarity of the obstructed cat liver was an unusual accumulation of phosphatase in capillaries, lymphatics, and in connective tissue surrounding the small and medium-sized bile ducts in the portal areas.

Following common duct ligation the glomeruli of the cat appeared to contain more alkaline phosphatase than normally, but owing to the dense stain of the normal glomeruli, it was difficult to be certain of this. Obstruction of the common duct in man and dog caused no demonstrable increase in the minimal glomerular activity normally present.

Cirrhosis of the Liver in Man

The distribution of the enzyme in liver lobules of Laennec's cirrhosis was considered of interest because the lesions indicated one or, in some cases, multiple ways in which the normal mechanism of excretion might be altered. In all 12 cases, due to regeneration, there was distortion of the normal anatomic relationships of the endothelial lining cells to the liver cells and to the canalicular system. Clinical evidence of abnormal liver function was present in all. Bile stasis was demonstrable in many

of the livers and in some there was liver cell necrosis. The well known fact that the excretion of phosphatase by the liver is usually not significantly impaired in cirrhosis is shown in Table VI. In 9 of 12 cases the serum level of enzyme was normal or only slightly elevated. In 7 livers in which the presence of bile plugs in the canaliculi suggested that bile stasis played a rôle in the mechanism of jaundice, the amount of alkaline phosphatase activity in the bile canaliculi did not exceed that seen in normal livers, and in only 2 of these cases was the serum enzyme level markedly elevated. This is illustrated in case 4 in which at autopsy canalicular bile plugs were abundant; bilirubin on the same day was 6.0 mg. per cent, but the phosphatase was only 6.8 Bodansky units per cent. In cases 5 and 6, the high serum levels of alkaline phosphatase appeared to indicate impaired excretion by the liver lobule. Histochemically, canalicular phosphatase was not increased abnormally but the endothelial sinus cells showed activity comparable to the high serum levels and to the activity seen in these cells in the cases of extrahepatic biliary obstruction. The cytoplasm of the liver cells showed no activity in any case in which they appeared anatomically normal, but in cases 1, 2, and 6 necrotic liver cells were strongly positive (Fig. 8). In the cases of cirrhosis without jaundice or necrosis of the liver cells, the amount of phosphatase and its distribution in the canaliculi and sinus lining cells were normal. In no cirrhotic liver was "rupture" of the bile canaliculi between liver cells demonstrated by enzyme activity as in regurgitation in extrahepatic biliary obstruction.

Subacute Yellow Atrophy of the Liver in Man

The liver of one patient with subacute yellow atrophy was studied (Fig. 9). Jaundice had been present for 9 weeks prior to death. Laboratory studies revealed serum alkaline phosphatase of 9.7 Bodansky units per 100 cc., bilirubin of 10.6 mg. per 100 cc., 4+ cephalin flocculation, and total cholesterol of 57 mg. per cent. There was extensive loss of liver parenchyma, involving all lobules in a haphazard fashion. Occasionally, liver cells were entirely destroyed and the stroma was collapsed. Remaining liver cells were in a poor state of preservation, the cytoplasm frequently including large, deep eosin-staining masses resembling colloid droplets. The cytoplasm of many liver cells contained clumps of bile pigment. No cause for the lesion could be determined.

Alkaline phosphatase activity was present in the cytoplasm of all liver cells. Activity was greater in those showing degeneration. Sinusoidal lining cells, bile canaliculi, and proliferating bile ducts and their lumina showed a mild degree of activity.

TABLE VI

Distribution of Alkaline Phosphatase in Cirrhotic Livers of Man

Case no.	Age	Liver lesions	Jaundice	Bile plugs (canaliculi)	Serum			Liver alkaline phosphatase				
					Alkaline phosphatase (B.U./100 cc.)	Bilirubin (mg./100 cc.)	Cephalin flocculation	Bile canaliculi	Sinusoidal lining cells	Liver cell cytoplasm	Portal connective tissue	Bile duct lumina
1	59	Laennec's cirrhosis; carcinoma of liver at hilum; obstruction of bile ducts; invasion of portal veins	+	++++	8.1 (13)*	23.0 (13)*	+++ (13)*	+	+	++†	++++	0
2	38	Laennec's cirrhosis	+	++++	8.2 (14)	12.7 (14)	+++ (14)	+	+	++†	++++	±
3	58	Laennec's cirrhosis; fatty liver	+	++	7.8 (2)	15.0 (2)	++ (2)	+	++	0	+++	±
4	31	Cirrhosis; intrahepatic pericholangitis	+	+++	6.8 (0)	6.0 (0)	++++ (0)	+	++	0	+++	0
5	63	Laennec's cirrhosis	+	++	15.0 (0)	8.0 (0)	++ (0)	+	+++	0	++++	±
6	41	Laennec's cirrhosis	+	++	14.0 (0)	19.0 (0)	++++ (0)	+	++++	++†	++	+
7	46	Laennec's cirrhosis	+	0	11.0 (6)	1.0 (6)	+++ (6)	±	+	0	+	0
8	64	Cirrhosis; intrahepatic pericholangitis	+	+	5.4 (0)	5.5 (0)	++ (0)	±	+	0	+	0
9	54	Laennec's cirrhosis	0	0	6.0 (0)	0.5 (0)	++++ (0)	±	+	0	+	±
10	44	Laennec's cirrhosis	0	0	2.8 (2)	2.0 (2)	++++ (2)	±	±	0	+	0
11	60	Laennec's cirrhosis	0	0	5.2 (0)	1.2 (0)	+++ (0)	+	+	0	++	0
12	30	Laennec's cirrhosis	0	0	4.3	0.5	-	-	±	0	++	0

* Figure in parentheses is the number of days before death when determination was made.
† Cells appeared autolyzed in routine sections.

DISCUSSION

In this semiquantitative determination of the alkaline phosphatase activity of the normal liver lobule and of the liver lobule of hepatocellular and obstructive jaundice, the problem of excretion has been considered in respect to the structures which separate circulating enzyme and bile canalicular enzyme.

We have been unable to demonstrate to our satisfaction the presence of alkaline phosphatase in the cytoplasm of the normal liver cell, and therefore have provided no direct evidence that the enzyme is excreted from the blood stream by the liver cells. In our experiments and in the human material, activity was demonstrated in the cytoplasm of damaged liver cells only. These findings are in agreement with some reports in the literature and are at variance with others.

Gomori,[10] in describing the liver cells of man, the dog, rabbit, and gopher, stated: "The liver cells are more or less positive in the same species." Takamatsu and Otsuki[11] found no activity in the liver cells of rats but noted that injured liver cells were positive. Kabat and Furth,[12] in studies of livers of man, chicken, and mouse, stated: "The bile ducts and liver cells contained no phosphatase, or only traces, although occasionally fine granules were seen scattered throughout the section but not identified with any cell type"; and in the same paper, "The observation that cells of a liver tumor induced in a rat by the feeding of butter yellow contained phosphatase was unexpected because liver and bile duct epithelium do not contain the enzyme." Bourne[13] found no activity in the liver cells of the guinea-pig. Wachstein and Zak[15] found that in sections incubated for 2 hours, the dog liver cell cytoplasm took on a grayish color and after 14 hours' incubation the staining of the cytoplasm was more intense. By prolonged incubation of sections they concluded that the activity in the cytoplasm of the obstructed dog liver was increased, but pointed out that "After longer incubation, the reaction became so intense that the structures could hardly be differentiated." Wachstein[14] found an increase in alkaline phosphatase activity in liver cells of mice, and to a lesser degree in rats, in starvation and protein depletion, but found no increase in these species when the liver cells were damaged experimentally by chloroform, carbon tetrachloride, or phosphorus. It is difficult to appraise the enzyme activity quantitatively in these experiments since the period of incubation of sections in the substrate mixture was varied from 8 to 12 hours and since specimens of the liver for comparison were not obtained before injury. In the study of one normal human liver, Wachstein and Zak[16] described the liver cells as staining faintly, with occasional cell groups showing more intense

reactions. Sections were incubated 12 to 14 hours. In obstructive jaundice the authors concluded that the liver cells showed little or no increase in alkaline phosphatase. Necrotic liver cells in subacute yellow atrophy and hepatitis were positive or showed increased activity.

Despite the evidence of little or no enzyme activity in the cytoplasm of liver cells in contrast to the abundance of activity in the bile canaliculi and adjacent sinus lining cells, it is difficult to believe that the enzyme does not pass through the liver cells to the bile. Lack of activity when the histochemical technic is employed cannot be used as evidence against hepatic cellular excretion, although good correlation is found between histochemical findings and chemical analyses of extracts of other tissues.[17] The enzyme may be present in too low a concentration to be demonstrable. It may be in an inactivated form, or may be inhibited by substances in the liver cell cytoplasm. Absence of demonstrable enzyme in the liver cells in biliary obstruction when the activity is increased strikingly in the canaliculi and sinus cells may be explained by the regurgitation of enzyme between the liver cells as clearly demonstrated by the connections between canaliculi and the sinus barrier.

The presence of similar but less common connections, as demonstrated by the histochemical technic, between the bile canaliculi and the sinus barrier of the normal liver lobule in man and the dog raises the question of the existence of a functional by-pass of the liver cell under physiologic conditions. Processes extending from the stellate or other endothelial lining cells between liver cells to the bile canaliculi have been described by von Kupffer[24] and by Pavel.[25] These observations are not widely accepted, but in view of the histochemical findings they might be reexamined. It may be speculated that such a by-pass could be an explanation both for the absence of the enzyme histochemically from the cytoplasm of the liver cell and for the fact that in hepatocellular jaundice the serum phosphatase may be normal or only very slightly elevated. Doubt is cast on such a hypothesis by the observation, in our cases of cirrhosis with little or no enzyme retention, that in no instance was a connection seen between bile canaliculi and the sinus barrier. Moreover, the fact that the enzyme may not be retained in the blood stream in hepatocellular jaundice in contrast to bilirubin may be due to differences in the ability of less damaged or uninjured portions of the liver lobule to excrete the two substances. Such increased excretion is suggested in the dogs in which the central third of the liver lobule was destroyed. Enzyme activity in endothelial cells and bile canaliculi in the uninjured portion of the lobule increased markedly.

The demonstration of the enzyme in the cytoplasm of the injured

liver cell raises the possibility that excretion of the enzyme may be altered in hepatocellular jaundice although retention is not appreciable. It is possible that the enzyme passes through the injured cell in larger quantities, thus becoming demonstrable by the histochemical technic. On the other hand, the necrotic liver cell may imbibe abundantly available enzyme from the bile or endothelial cells. Another explanation might be that enzyme present normally in the cytoplasm and not demonstrable by the histochemical technic is activated by the intracellular changes attendant upon necrosis. Wachstein and Zak[16] have suggested that the enzyme may be concentrated in shrunken, injured liver cells. In our cases of acute liver cell degeneration in cirrhosis in which the activity in the cytoplasm was great, the liver cells were not shrunken.

In these observations it has been found that the concentration of enzyme activity in the endothelial cells lining the hepatic sinuses increases and decreases in the same direction as the activity in the blood stream. It would seem likely, then, that a function of these cells is to transmit the enzyme to and from the blood. In obstruction, the marked increase in endothelial cellular activity may be due to regurgitation from the bile through these cells. However, a by-pass of these cells by lymphatic drainage of the space of Disse is another retrograde pathway to be considered. That these cells may be actively concerned with regulating the serum enzyme level is suggested by the increase in their activity when other portions of the liver lobule were destroyed, as observed in the dog.

The futility of attempting to compare the retention of bile pigments and phosphatase is illustrated by the findings in the cirrhotic livers. In 7 jaundiced patients, the presence of bile plugs in the biliary canaliculi at autopsy indicated that the bilirubinemia was in part due to bile stasis. Under such circumstances, retention of phosphatase might be expected, but the serum level was elevated in only 2 cases. In these and in all the other cases there was no increase in canalicular phosphatase such as was seen in biliary stasis without cirrhosis.

SUMMARY

Alkaline phosphatase activity determined semiquantitatively in the normal liver of man, the dog, and the cat is demonstrable in cells lining the hepatic sinuses, in the bile canaliculi, in the nuclei of liver cells, and inconstantly in the epithelium of intrahepatic bile ducts. No activity is demonstrable in the cytoplasm of normal liver cells in these species. Branch bile canaliculi delineated by the results of enzyme activity occasionally separate liver cells and extend to the sinus barrier.

Following obstruction of the bile ducts in man and the dog, the

alkaline phosphatase increases in the bile canaliculi and in the cells lining the sinuses but is not demonstrable in the cytoplasm of the liver cells. Regurgitation of the enzyme between liver cells is demonstrated microchemically in biliary obstruction. The increase in enzyme activity in the bile canaliculi and endothelial cells, and in the serum following obstruction of the common duct, is less conspicuous in the cat, in which the renal glomeruli show great enzyme activity in contrast to the glomeruli of man and dog.

Alkaline phosphatase is demonstrable in the cytoplasm of liver cells in the following instances: an occasional necrotic liver cell in biliary obstruction in man; (b) necrotic liver cells in experimental chloroform and carbon tetrachloride poisoning of dogs; (c) in both histologically normal and necrotic liver cells in a case of subacute yellow atrophy in man; and (d) in liver cells showing changes usually ascribed to autolysis in cirrhosis of the liver of man with jaundice.

Alkaline phosphatase activity is increased in the bile canaliculi and sinus lining cells of the livers of dogs in which serum alkaline phosphatase, transiently elevated following liver injury by chloroform, is falling to normal.

Following complete destruction by carbon tetrachloride of the liver cells in the central zones of the liver lobules of dogs, the serum alkaline phosphatase is elevated moderately and the enzyme activity is increased in the sinus lining cells and in the bile canaliculi in the undamaged portion of the lobule.

The enzyme activity of the sinus lining cells and of the bile canaliculi of human cirrhotic liver is not increased when the serum activity is increased or when it is normal. In human cases of hepatic cirrhosis showing evidence of intrahepatic biliary stasis and high serum alkaline phosphatase activity, the enzyme activity of the sinus lining cells is increased as in other conditions with elevated serum levels, but the activity of the bile canaliculi is normal in contrast to the increase in activity in biliary obstruction without cirrhosis.

REFERENCES

1. Armstrong, A. R., King, E. J., and Harris, R. I. Phosphatase in obstructive jaundice. *Canad. M. A. J.*, 1934, **31**, 14–20.

2. Bodansky, A., and Jaffe, H. L. Phosphatase studies. VIII. Increase of serum phosphatase after bile duct ligation in dog. *Proc. Soc. Exper. Biol. & Med.*, 1933–34, **31**, 1179–1181.

3. Armstrong, A. R., and Banting, F. G. The site of formation of the phosphatase of serum. *Canad. M. A. J.*, 1935, **33**, 243–246.

4. Maddock, S., Schmidt, G., and Thannhauser, S. J. The effect of the intravenous injection of alkaline intestinal phosphatase on dogs following hepatectomy and total abdominal evisceration with and without hepatectomy. *Federation Proc.*, 1942, **1**, 181.

5. Freeman, S., Chen, Y. P., and Ivy, A. C. On the cause of the elevation of phosphatase in jaundice. *J. Biol. Chem.*, 1938, **124**, 79–87.

6. Roberts, W. M. Variations in the phosphatase activity of the blood in disease. *Brit. J. Exper. Path.*, 1930, **11**, 90–95.

7. Bodansky, A., and Jaffe, H. L. Phosphatase studies. IV. Serum phosphatase of non-osseous origin. Significance of the variations of serum phosphatase in jaundice. *Proc. Soc. Exper. Biol. & Med.*, 1933–34, **31**, 107–109.

8. Flood, C. A., Gutman, E. B., and Gutman, A. B. Phosphatase activity, inorganic phosphorus and calcium of serum in disease of liver and biliary tract. *Arch. Int. Med.*, 1937, **59**, 981–999.

9. Gutman, A. B., Olson, K. B., Gutman, E. B., and Flood, C. A. Effect of disease of the liver and biliary tract upon the phosphatase activity of the serum. *J. Clin. Investigation*, 1940, **19**, 129–152.

10. Gomori, G. The distribution of phosphatase in normal organs and tissues. *J. Cell. & Comp. Physiol.*, 1941, **17**, 71–83.

11. Takamatsu, H., and Otsuki, T. Untersuchungen über die Phosphatasereaktion der Leberzellen der normalen und der mit o-Amidoazotoluol-Diät gefütterten Ratten. *Gann*, 1941, **35**, 283.

12. Kabat, E. A., and Furth, J. A histochemical study of the distribution of alkaline phosphatase in various normal and neoplastic tissues. *Am. J. Path.*, 1941, **17**, 303–318.

13. Bourne, G. The distribution of alkaline phosphatase in various tissues. *Quart. J. Exper. Physiol.*, 1943–44, **32**, 1–19.

14. Wachstein, M. Influence of dietary deficiencies and various poisons on the histochemical distribution of phosphatase in the liver. *Arch. Path.*, 1945, **40**, 57–67.

15. Wachstein, M., and Zak, F. G. Histochemical distribution of alkaline phosphatase in dog liver after experimental biliary obstruction. *Proc. Soc. Exper. Biol. & Med.*, 1946, **62**, 73–76.

16. Wachstein, M., and Zak, F. G. Distribution of alkaline phosphatase in the human liver. *Arch. Path.*, 1946, **42**, 501–516.

17. Gomori, G. Microtechnical demonstration of phosphatase in tissue sections. *Proc. Soc. Exper. Biol. & Med.*, 1939, **42**, 23–26.

18. King, E. J., and Armstrong, A. R. A convenient method for determining serum and bile phosphatase activity. *Canad. M. A. J.*, 1934, **31**, 376–381.

19. Bodansky, A. Phosphatase studies. II. Determination of serum phosphatase. Factors influencing the accuracy of the determination. *J. Biol. Chem.*, 1933, **101**, 93–104.

20. Bodansky, A. Non-osseous origins of serum phosphatase: the liver. *Enzymologia*, 1937, **3**, 258–260.

21. Cantarow, A., Stewart, H. L., and McCool, S. G. Serum phosphatase in cats with total bile stasis. *Proc. Soc. Exper. Biol. & Med.*, 1936–37, **35**, 87–89.

22. Flood, C. A., Gutman, E. B., and Gutman, A. B. Serum and urine phosphatase activity in the cat after ligation of the common bile duct. *Am. J. Physiol.,* 1937, **120,** 696–702.

23. Cantarow, A. The influence of icteric sera with high phosphatase activity on the phosphatase activity of normal sera. *Am. J. Clin. Path.,* 1940, **10,** 858–863.

24. von Kupffer, C. Ueber die sogenannten Sternzellen der Säugethierleber. *Arch. f. mikr. Anat.,* 1899, **54,** 254–288.

25. Pavel, I. Les ictères. Bucharest, 1943, p. 113.

[*Illustrations follow*]

DESCRIPTION OF PLATES

PLATE 173

All illustrations have been made from alkaline phosphatase preparations.

FIG. 1. Normal liver of man. Enzyme activity is represented by the dark granules in the lining cells of the sinuses and in the bile canaliculi. No activity is seen in the cytoplasm of the liver cells.

FIG. 2. Liver of man after common duct obstruction. The phosphatase activity of sinus cells and bile canaliculi is increased in contrast to the activity in normal liver. The extension of the canaliculi to the sinus cells indicates rupture. Of note is the absence of granules in the cytoplasm of the liver cells.

FIG. 3. Normal liver of dog. Phosphatase activity in the bile canaliculi and sinus cells is greater than in the normal liver of man.

FIG. 4. Liver of the dog after ligation of the common duct. Increased enzyme activity is shown in the sinus cells and bile canaliculi.
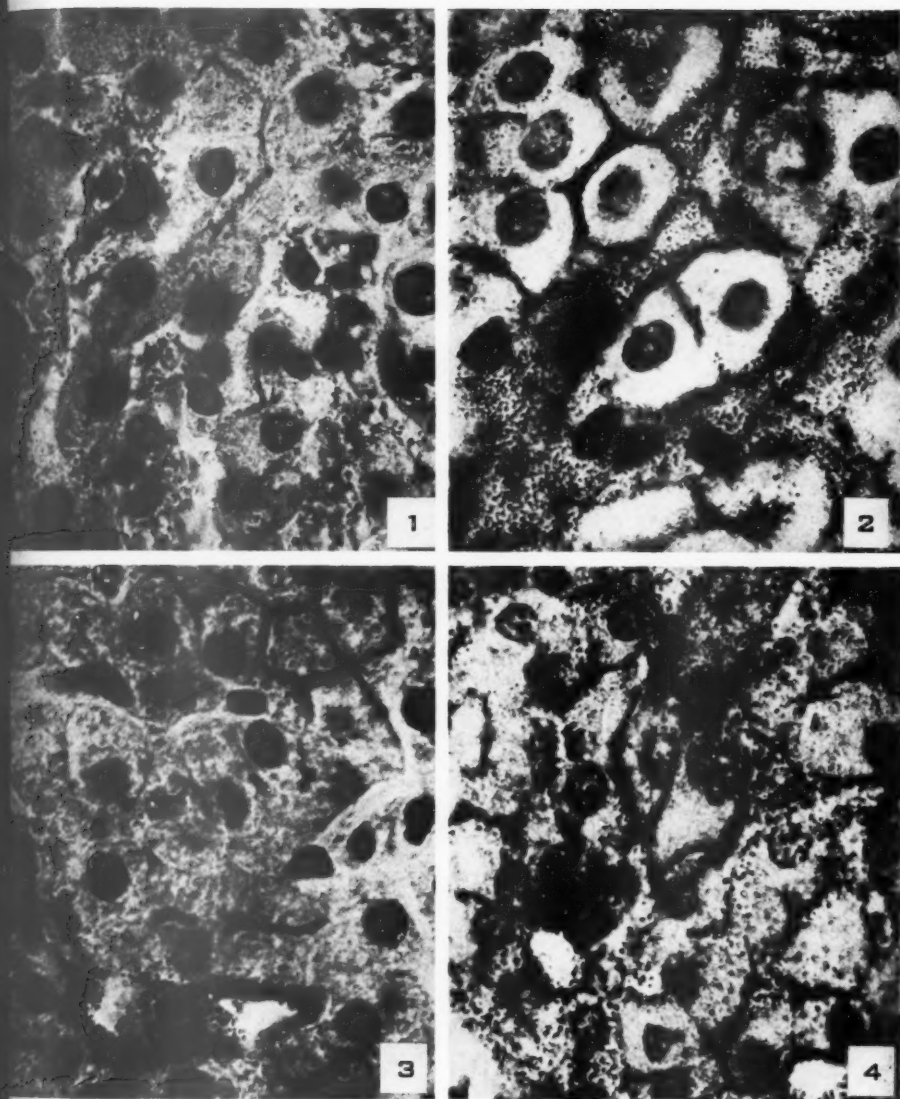
Kritzler and Beaubien

Alkaline Phosphatase Activity of Liver

PLATE 174

FIG. 5. Liver of dog, showing complete destruction of the liver cells of the central zones due to carbon tetrachloride. No evidence of activity is seen in the central zone (lower right). Enzyme activity of the bile canaliculi and sinus cells in the normal portion of the liver lobule is greater than is normal.

FIG. 6. Normal kidney of the dog. The enzyme activity of the glomerulus is slight.

FIG. 7. Normal kidney of the cat. The glomerulus is blackened by granules, indicating strong phosphatase activity.
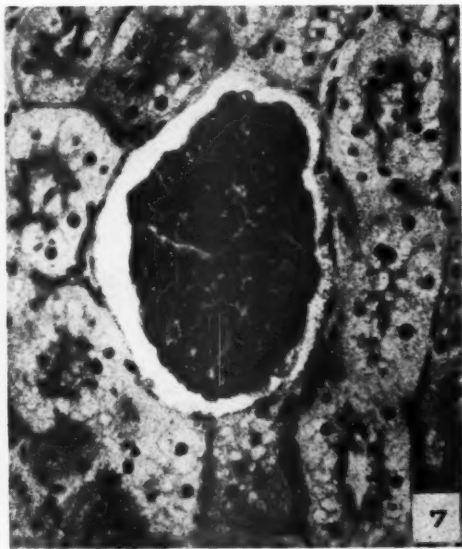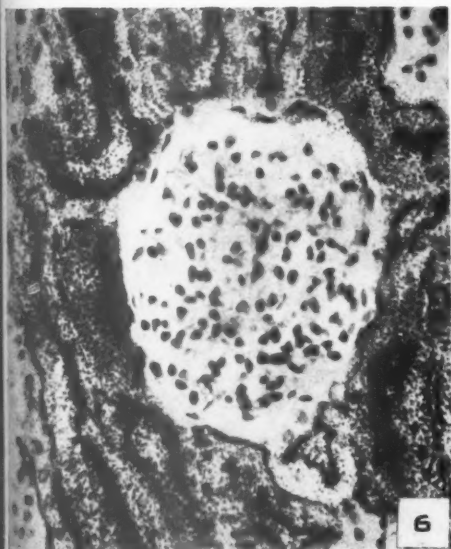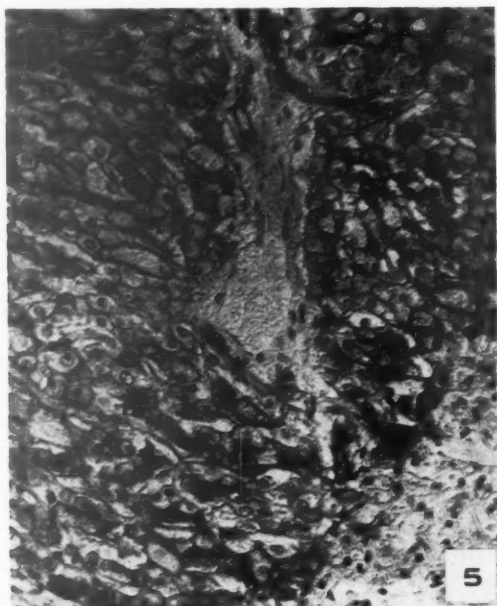
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Alkaline Phosphatase Activity of Liver

PLATE 175

FIG. 8. Human hepatic cirrhosis with jaundice. There is striking activity in the cytoplasm of liver cells which appeared to be autolyzed in ordinary sections.

FIG. 9. Subacute yellow atrophy in a human liver. The cytoplasm of all liver cells is stippled by granules, indicating enzyme activity.
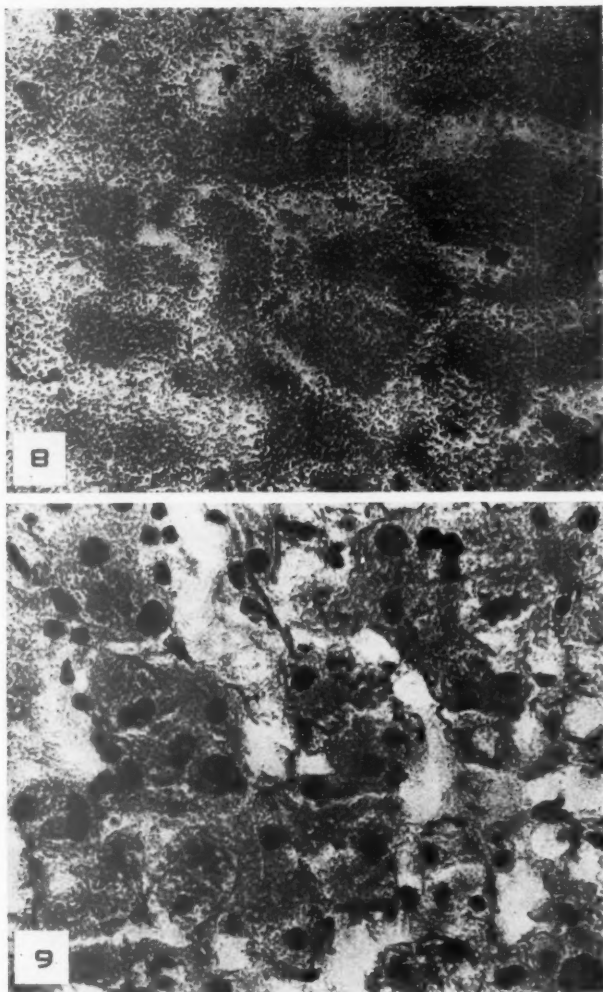
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Kritzler and Beaubien   Alkaline Phosphatase Activity of Liver

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THE ISOLATION OF MUMPS VIRUS AT AUTOPSY[*]

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In 1934 Johnson and Goodpasture[1] demonstrated that mumps was caused by a filterable virus and showed that the rhesus monkey could be infected experimentally. Their observations and those of subsequent workers on the pathologic changes in the parotid gland of the monkey provided the first opportunity for the specific correlation of the etiologic agent with these changes. To date, similarly authenticated observations have been lacking on human material. The development by Enders and Cohen[2] of a specific serologic test for mumps, and the propagation of mumps virus in the developing egg by Habel[3] and Levens and Enders[4] have greatly stimulated clinical and laboratory studies on this entity. Using these newly developed technics, information has been obtained on the distribution of the virus in certain body fluids and secretions.[5-7] An opportunity to obtain further information on this subject was afforded when a patient with Sturge-Weber syndrome and pneumonia died with a concurrent mumps infection. The present paper reports the isolation of mumps virus from the tissues of this patient, a description of the specific pathologic changes thus authenticated, and the general pathologic findings.

Clinical History and Course

E. F., a 9-year-old female, had been studied at the Children's Hospital since the age of 7½ months because of frequent psychomotor and grand mal attacks. The presence of a hemangioma involving the left periorbital region and the demonstration of cerebral calcification suggested the diagnosis of Sturge-Weber syndrome.

Approximately 70 hours before death, the patient awoke at night complaining of soreness in the preauricular area. The following morning her mother thought she had localized preauricular swelling and kept her home from school. At noon, the patient complained of malaise and shortly thereafter had a brief grand mal attack. A second grand mal attack, which lasted several hours and was followed by deep coma, occurred approximately 50 hours before death. Thirty-six hours before death, she was admitted to the Children's Hospital. On admission, the patient was comatose and did not respond to painful stimuli. (Temperature, 103° F.; pulse, 74; respirations, 40; blood pressure, 110/65 mm. of Hg.) A large hemangioma was present on the left cheek and forehead. The left pupil was larger than the right and did not react to light. The fundi were normal except for tortuosity of the vessels around the left disk. No definite parotid enlargement was observed. The corneal and pharyngeal reflexes were absent. The lungs were normal to percussion and auscultation; examination of the heart revealed no significant findings. The deep tendon reflexes were hypoactive and Babinski's and Oppenheim's signs were positive bilaterally.

Lumbar puncture on admission showed a pressure of 130 mm. of water and yielded clear fluid containing 256 red blood cells per cmm. and no white cells; the protein was 24 mg. per cent, the sugar was slightly decreased by qualitative test, and there was no growth on culture. Blood studies revealed a white blood count of 13,800 with 85 per cent polymorphonuclear cells, 11 per cent lymphocytes, and 4 per cent monocytes. The blood nonprotein nitrogen was 103 mg. per cent and the sugar, 35 mg. per cent. Blood cultures on admission yielded no growth.

A constant intravenous drip of 10 per cent glucose in water was started, followed by normal saline solution; 30,000 units of penicillin was administered every 4 hours. At the end of the first 24 hours in the hospital the blood sugar was 112 mg. per cent and the lumbar puncture yielded fluid containing 139 red cells and 10 white cells per cmm. of which 9 were mononuclear. Swelling of both parotid regions had become apparent. The patient remained unconscious and again began to have intermittent generalized clonic convulsions. Sodium phenobarbital, 0.195 gm., was given intravenously. An hour later the child appeared cyanotic, the heart sounds became inaudible, and the respirations ceased shortly thereafter. During the 36-hour hospital stay the temperature remained between 102.2° and 105.4°F. A third lumbar puncture done 5 hours before death yielded clear fluid with 180 red and 5 white cells per cmm.

Post-mortem Findings

Post-mortem examination (A48-54) was performed 9 hours after death. In the following description only the significant findings are included; the encephalotrigeminal angiomatous process is not described in detail in the present report.

Gross Description. Gross examination revealed a purple, sharply delineated, flat, vascular nevus involving the left forehead and upper eyelid. The preauricular regions were not swollen. The cut surfaces of the lungs were moist, but no marked edema was present. The pancreas showed no gross abnormality. On sectioning, the liver revealed a striking yellowish brown coloration in the periphery of the lobules with a darker purple in the central areas; the latter were slightly depressed. The ovaries showed no evidence of hemorrhage or injection; their external surfaces were smooth. The right parotid gland, only, was exposed and sectioned; it did not appear edematous nor were any petechiae seen. The cranium was not thickened and did not display an abnormal vascular pattern. The dura was normal. On the right side the cerebral veins were not remarkable. The left cerebral hemisphere was smaller than the right, and this difference became progressively more marked toward the occipital pole. The entire inferior lateral aspect of the left parietal and occipital hemispheres was overlaid by a thin, dark-red, membranous covering; this on close examination was seen to be made up of a mattress of interlacing meningeal vessels which obscured the convolutions and the sulci. The basal ganglia, the brain stem, and cerebellum were normal. No areas of hemorrhage or infarction were noted in the brain.

Microscopic Description. Tissues were fixed in Zenker's acetic acid

solution or 10 per cent neutral formalin and were stained routinely with hematoxylin and eosin; other special stains were employed as noted below.

In sections from all portions of the lungs there was an acute lobular pneumonia, the majority of the alveoli being filled with a polymorphonuclear exudate. The bronchioles contained moderate amounts of purulent material but the distribution of the inflammatory exudate was not bronchopneumonic in pattern. With the Wolbach modification of the Giemsa and the Lerner-Gram* stains only occasional bacteria were found; these occurred both intracellularly and extracellularly. The spleen contained a few reaction centers in the malpighian corpuscles. The pancreas showed no degenerative or inflammatory alteration. The liver was the seat of an unusual and marked degenerative change. The general architecture of the liver cords remained and no collapse of reticulum was noted after use of Foot's reticulum stain. The parenchymal cells, except in the extreme periphery of the hepatic lobules, stained bright red with eosin, although the Kupffer cells retained the basic dyes. The nuclei of the liver cells stained poorly or not at all, but there was no disintegration of the cell membranes. The parenchymal cells throughout the lobules were filled with fat (scharlach R stain) and this was especially marked in the central areas. No inflammatory cell invasion or evidence of regeneration was observed. The distal convoluted and collecting tubules of the kidney contained numerous hyaline casts; the proximal tubular cells were swollen, many having an increased amount of stainable lipids. The ovary was slightly congested and there was an occasional lymphocyte in the central stroma.

The general architecture of the parotid gland (Figs. 1 and 2) was well preserved, the major alterations in structure occurring in and about the ducts which showed widespread involvement. In the least affected ducts, there was a slight swelling of the epithelial lining cells with an increased granularity of their cytoplasm. The lumina contained a small amount of débris consisting of nuclear fragments, polymorphonuclear leukocytes, and desquamated epithelial cells. In the more severely involved ducts, a large portion of the ductal epithelium had desquamated, leaving a bare basement membrane. Other epithelial cells were considerably swollen and their nuclei pushed to the side of the cell. A rare basophilic, irregularly outlined, cytoplasmic inclusion body could be seen; these filled one-third to two-thirds of the total cell volume. In the most severely involved region, there was great dilatation of the ducts; all

Lerner, E. M. A rapid Gram stain for tissue. *Arch. Path.*, 1946, 41, 674-675.

of the epithelial cells were desquamated and a large mass of nuclear and cellular débris choked the lumen. The inflammatory reaction spread for a short distance out into the periductal connective tissues and tangentially involved the adjacent acini. All of the acini at a distance from the ductal structures were completely normal. There was a minor inflammatory reaction in perineurium in one area of the capsular structure; it consisted of a few lymphocytes and monocytes. The interstitial exudate contained little or no fibrin, yet the periductal edema was moderate to marked in degree. In the periductal regions, the lymphocyte was the predominant inflammatory cell; a few large mononuclear and rare polymorphonuclear cells were present. The difference in the inflammatory cell types without and within the ducts was striking.

The brain away from the angiomatous areas appeared normal. No congestion, edema, or hemorrhage, nor inflammatory or degenerative change was seen.

SUMMARY OF VIRUS ISOLATION AND SEROLOGIC STUDIES

Materials Available and Method of Storage

Blood. One sample of blood was obtained 36 hours before death. This was kept at 5°C. for 48 hours and then the serum was separated and stored at —25°C.

Cerebrospinal Fluid. Two specimens of cerebrospinal fluid were obtained, consisting of sample A collected 36 hours before death, and sample B collected at autopsy 9 hours after death. Both specimens were kept at 5°C. for 48 hours and then placed in the CO_2 (dry ice) box in sealed glass ampules.

Tissues. The following specimens were obtained at autopsy: 5 gm. of small intestine; 1.5 gm. of the upper pole of the right parotid gland; 1.5 gm. of spinal cord from the thoracic region; 6.4 gm. of cerebral cortex from the right parietal area; 3.5 gm. of spleen; and one-half of each ovary, total weight 1.5 gm. The segment of small intestine was opened and washed in two changes of normal saline solution. Individual 10 per cent suspensions by weight of the various tissues were then prepared in isotonic phosphate buffer (pH 7.2) by grinding with alundum. To 9.4 cc. of each organ suspension, 0.6 cc. of a solution of penicillin and streptomycin were added so that each 1 cc. of the resulting mixture contained 500 units of penicillin G and 0.5 mg. of streptomycin base. The suspensions were then centrifuged for 5 minutes at 1000 r.p.m.; the supernatants were removed, sealed in glass ampules, and stored in the CO_2 ice box.

Isolation and Serologic Identification of an Agent from the Parotid Gland

After storage for 2 days, 0.1 cc. of the 10 per cent parotid gland suspension was inoculated intra-amniotically, as previously described,[8] into 5-day old embryonated hen's eggs. The amniotic fluids were harvested from seven eggs after 6 days' incubation at 35°C.; four of the seven fluids contained hemagglutinins for hen's cells in a titer of 1:8 or greater when tested by a modified Salk technic.[8] A second amniotic passage was done and the average hemagglutinin titer of the fluids when harvested 5 days later was found to be 1:512. Identification studies on the hemagglutinating agent were carried out, employing pooled amniotic fluid from the third egg passage, hereafter termed the EF strain. Complement-fixation tests were done by the technic employed in this laboratory.[8] In these tests, the EF strain and amniotic fluid harvested from the 42nd egg passage of a stock strain of mumps virus were employed as antigens. The serums used included specimens collected during the acute and convalescent phases of mumps from experimentally infected monkeys, from human cases of mumps, and that from patient EF obtained 36 hours before death. The data are presented in Table I. Comparable results were obtained with the two antigens, although there was some variation in titers.

TABLE I

Results of Complement-Fixation Tests Employing the EF and Stock Strain of Mumps Virus

Antigen	Serum	Reciprocal of initial dilution of serum					
		8	32	128	512	2048	8192
EF	Acute monkey 148	2	tr	o	nd	nd	nd
Stock	Acute monkey 148	o	o	o	nd	nd	nd
EF	Convalescent monkey 1390	4	4	4	tr	tr	tr
Stock	Convalescent monkey 1390	4	4	4	tr	tr	tr
EF	Acute human 132	4	3	2	tr	nd	nd
Stock	Acute human 132	4	1	tr	o	nd	nd
EF	Convalescent human 132	4	4	4	4	1	tr
Stock	Convalescent human 132	4	4	4	3	tr	o
EF	Acute human 134	nd	4	3	tr	nd	nd
Stock	Acute human 134	3	1	tr	tr	nd	nd
EF	Convalescent human 145	4	4	4	2	tr	tr
Stock	Convalescent human 145	4	4	4	4	4	tr
EF	Patient EF	4	4	2	tr	tr	o
Stock	Patient EF	2	tr	tr	o	o	o

4 = complete fixation of complement.
tr = minimal detectable fixation of complement.
nd = not done.

Agglutinin-inhibition tests were similarly carried out employing the same antigens as above and comparable serums. The technic used was a further modification of the Salk method, which increases the specificity

of the test.[8,9] Two hemagglutinating units of virus were used and the virus-serum mixtures maintained overnight at 5°C. prior to the addition of hen's cells. The results of the tests are summarized in Table II. From all of these observations it is apparent that the agent isolated from the parotid gland behaves as does mumps virus in respect to its growth in eggs, and its hemagglutinative and complement-fixing properties.

Titration of the Egg Infectivity of the Parotid Gland

Titration of the infectivity of the parotid gland was carried out after storage for 21 days by inoculating groups of eight eggs with falling dilutions from 10^{-1} to 10^{-6} of the suspension made up in a buffer. The amniotic fluids were harvested and tested individually for hemagglutinins after 6 days' incubation. The fluid from one of seven surviving eggs inoculated with the lowest dilution gave a positive test, while the eggs inoculated with higher dilutions were negative. (On subsequent passage of the material from the single positive egg, the agent exhibited the properties of mumps virus.) These findings indicate that the infective titer of the parotid gland suspension was low, although the possibility cannot be eliminated that inactivation of an unknown proportion of the virus occurred during the period of storage.

Isolation of Mumps Virus from Other Organs

Attempts were made to isolate a virus from the other organ suspensions after they had been stored for 12 to 25 days. Spleen, intestine, spinal cord, brain, and the two specimens of spinal fluid were each inoculated intra-amniotically into eggs and carried for 3 or 4 passages; no hemagglutinating agent was isolated. The suspensions of pancreas and ovary were handled similarly; on the second egg passage, mumps virus was isolated from one of these, but owing to a laboratory error it was impossible to ascertain from which of the two organs the virus had been obtained. Subsequently, three additional attempts to isolate the virus from the suspensions of ovary and pancreas as well as additional attempts to isolate a virus from the other organs failed. Parotid gland material removed from infected monkeys at an appropriate stage of the disease has proved to be a satisfactory complement-fixing antigen.[2] Therefore, search was made for the presence of a similar antigen in suspensions of the parotid gland, spleen, and pancreas from the present case; no specific fixation of complement could be detected.

DISCUSSION

With the development of technics for the isolation of mumps virus in eggs, it has been demonstrated that the virus can be isolated with rela-

TABLE II

TABLE II

Result of Agglutinin-Inhibition Tests Employing the EF and Stock Strain of Mumps Virus

Antigen	Serum	Reciprocal of final dilution of serum													
		8	16	32	64	128	256	512	1024	2048	4096	8192	16384	32768	65536
EF strain	Acute monkey 2	—	—	±A	±A	±A	±A	±A	+	+	+	+	+	±	+
EF strain	Convalescent monkey 2	—	—	±	±	±	±	±	±	—	—	—	—	±	±
EF strain	Acute monkey 1390	—	—	—	—	—	—	±	+A	+	+	+	+	+	+
EF strain	Convalescent monkey 1390	—	—	—	—	±	±	±	±	—	—	—	—	+	+
Stock mumps	Acute monkey 1390	—	—	—	—	—	—	±	+A	+	+	+	+	±	+
Stock mumps	Convalescent monkey 1390	—	—	—	—	±	±	±	±	—	—	—	—	±	+
EF strain	Acute human 118	—	—	—	—	—	±	+	+	+	+	+	+	+	+
EF strain	Convalescent human 118	—	—	—	—	—	±	±	+	+	+	±	+	±	+
Stock mumps	Acute human 118	—	—	—	—	—	—	±	+	+	+	+	+	+	+
Stock mumps	Convalescent human 118	—	—	—	—	±	±	±	±	—	—	—	—	—	±

+ = Complete agglutination of red blood cells.
± = Incomplete agglutination of red blood cells.
— = No agglutination of red blood cells.
A = Atypical pattern of agglutinated cells.

tive ease directly from the saliva of man during the first few days of the acute illness.[5] Virus has also been isolated in eggs from parotid material of the experimentally infected monkey,[3,4] and the pathologic changes in experimentally infected monkeys have been described.[10-12] As far as is known, mumps virus has not been isolated heretofore from the human parotid gland. While a few descriptions of the pathologic changes in the human parotid gland have appeared in the literature, in no case has the diagnosis been established on other than clinical grounds.

Previous reports on the gross lesions of the human parotid gland in mumps have described enlargement of the gland with edema, congestion, and scattered petechial hemorrhages,[13-16] and similar alterations have been observed in the monkey. In the material obtained from the present case no gross changes were apparent.

The microscopic findings in the parotid gland of the present case consisted essentially of periductal interstitial edema, degeneration of the ductal epithelium with subsequent polymorphonuclear infiltration, and a mononuclear response in the interstitial tis-

sues. These changes in general correspond closely to those previously described in man,[17-20] as well as the monkey, and are therefore confirmatory of the earlier descriptions. However, the extensive acinar degenerative changes observed in the experimental animal[10] and by de Lavergne et al.[18] in man were not seen in the present case.

The recent isolation of mumps virus from the spinal fluid[6,7] and the blood[7] in patients with mumps demonstrates that, on occasion at least, the virus has a generalized distribution in the body. In the present case, although there was no specific gross or microscopic evidence of involvement of organs other than the parotid gland, virus was isolated from either the pancreas or the ovary. It appears doubtful that the acute necrotic process in the liver was specifically related to the mumps virus, since it could be explained on the basis of the extensive pneumonia and associated anoxemia; however, there is one somewhat questionable report in the literature of acute hepatic degeneration associated with mumps.[21] Unfortunately, no liver tissue was saved for virus isolation studies. No pathologic or experimental evidence of involvement of the central nervous system by the virus was obtained in this case; this aspect is reviewed by Donohue.[19]

SUMMARY

An agent, shown to be similar to a known strain of mumps virus in its biologic and immunologic properties, was isolated at autopsy from the tissues of a patient with the Sturge-Weber syndrome, pneumonia, and mumps infection. The virus was found to be present in comparatively low concentration in suspensions of the parotid gland. It was also isolated from either the pancreas or the ovary. No virus could be demonstrated in specimens of spinal fluid.

Pathologic changes which could be attributed to the effect of the mumps virus were limited to the parotid gland and consisted of periductal interstitial edema, a mononuclear response in the interstitial tissues, and degeneration of the ductal epithelium with a polymorphonuclear infiltration. These changes are similar to those previously described in mumps parotitis in man and in the experimentally infected monkey.

REFERENCES

1. Johnson, C. D., and Goodpasture, E. W. An investigation of the etiology of mumps. *J. Exper. Med.*, 1934, **59**, 1–19.
2. Enders, J. F., and Cohen, S. Detection of antibody by complement-fixation in sera of man and monkey convalescent from mumps. *Proc. Soc. Exper. Biol. & Med.*, 1942, **50**, 180–184.

3. Habel, K. Cultivation of mumps virus in the developing chick embryo and its application to studies of immunity to mumps in man. *Pub. Health Rep.*, 1945, 60, 201–212.

4. Levens, J. H., and Enders, J. F. The hemoagglutinative properties of amniotic fluid from embryonated eggs infected with mumps virus. *Science*, 1945, 102, 117–120.

5. Leymaster, G. R., and Ward, T. G. Direct isolation of mumps virus in chick embryos. *Proc. Soc. Exper. Biol. & Med.*, 1947, 65, 346–348.

6. Henle, G., and McDougall, C. L. Mumps meningo-encephalitis. Isolation in chick embryos of virus from spinal fluid of a patient. *Proc. Soc. Exper. Biol. & Med.*, 1947, 66, 209–211.

7. Kilham, L. Isolation of mumps virus from the blood of a patient. *Proc. Soc. Exper. Biol. & Med.*, 1948, 69, 99–100.

8. Enders, J. F., and Levens, J. H. Mumps. In: Diagnostic Procedures for Virus and Rickettsial Diseases. American Public Health Association, New York, 1948, pp. 139–164.

9. Robbins, F. C., Kilham, L., Levens, J. H., and Enders, J. F. An evaluation of the test for antihemagglutination in the diagnosis of infections by the mumps virus. *J. Immunol.*, 1949, 61, 235–242.

10. Johnson, C. D., and Goodpasture, E. W. The histopathology of experimental mumps in the monkey, *Macacus rhesus*. *Am. J. Path.*, 1936, 12, 495–510.

11. Findlay, G. M., and Clarke, L. P. The experimental production of mumps in monkeys. *Brit. J. Exper. Path.*, 1934, 15, 309–313.

12. Bloch, O., Jr. Specificity of the lesions of experimental mumps. *Am. J. Path.*, 1937, 13, 939–944.

13. Bamberger, H. Die Krankheiten der Mund- und Rachenhöhle, der Parotis, des Halszellgewebes und des Oesophagus. In: Virchow, R. Handbuch der speciellen Pathologie und Therapie. F. Enke, Erlangen, 1864, 6, Pt. 1, 60–67.

14. Delater. Les lésions histologiques des glandes salivaires dans les oreillons. *Ann. de méd.*, 1922, 11, 503–508.

15. Lang, F. J. Pathologische Anatomie der grossen Kopfspeicheldrüsen. In: Henke, F., and Lubarsch, O. Handbuch der speziellen pathologischen Anatomie und Histologie. J. Springer, Berlin, 1929, 5, Pt. 2, 47–49.

16. Rocchi, F. Anatomia patologica della parotite epidemica. *Pathologica*, 1933, 25, 690–704.

17. Dopter and Repaci, G. Contribution à l'étude anatomo-pathologique des oreillons. *Arch. de méd. expér. et d'anat. path.*, 1909, 21, 533–545.

18. de Lavergne, V., Kissel, P., and Leichtman, P. Les lésions de la parotidite ourlienne. *Presse méd.*, 1939, 47, 961–963.

19. Donohue, W. L. The pathology of mumps encephalitis with report of a fatal case. *J. Pediat.*, 1941, 19, 42–52.

20. Decloux, L., and Dumas, L. R. In: Cornil, V., and Ranvier, L. Manuel d'histologie pathologique. F. Alcan, Paris, ed. 3, 1912, 4, 419.

21. Lemoine, G. H., and Lapasset, F. Un cas de pancréatite ourlienne avec autopsie. *Bull. méd.*, Paris, 1905, 19, 617–620.

[*Illustrations follow*]

DESCRIPTION OF PLATE

PLATE 176

Fig. 1. Parotid gland showing ductal and interstitial involvement. Wolbach's modification of the Giemsa stain. × 175.

Fig. 2. Higher magnification of an area in the parotid gland showing the polymorphonuclear cellular response in the lumen of a duct and the mononuclear infiltration around the duct. Wolbach's modification of the Giemsa stain. × 500.
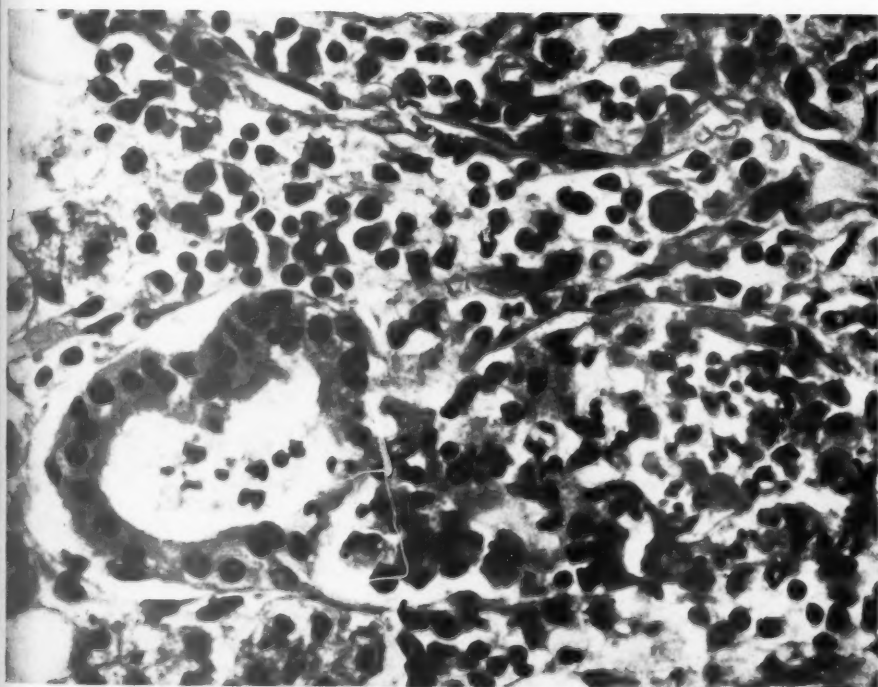
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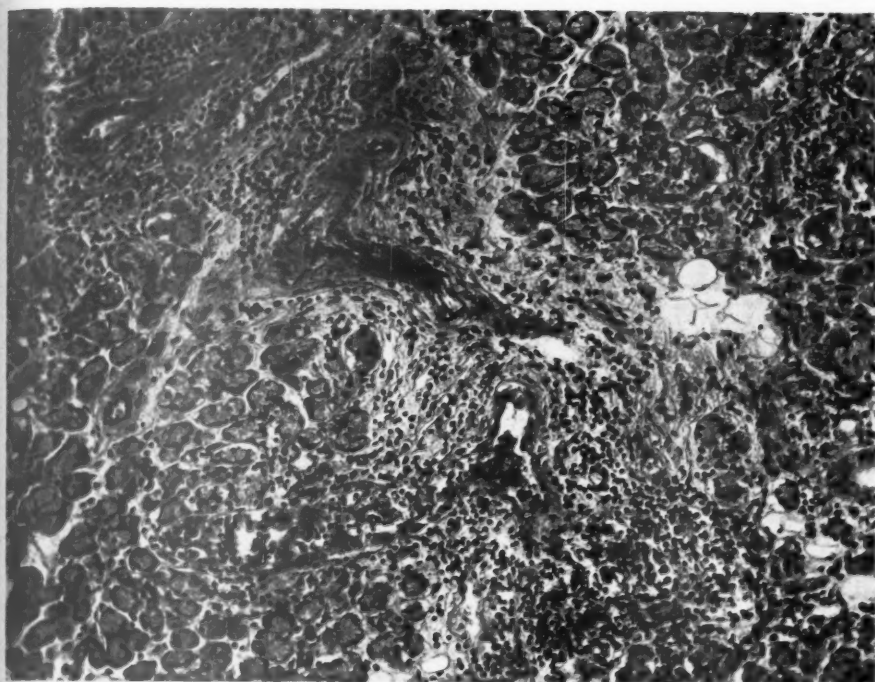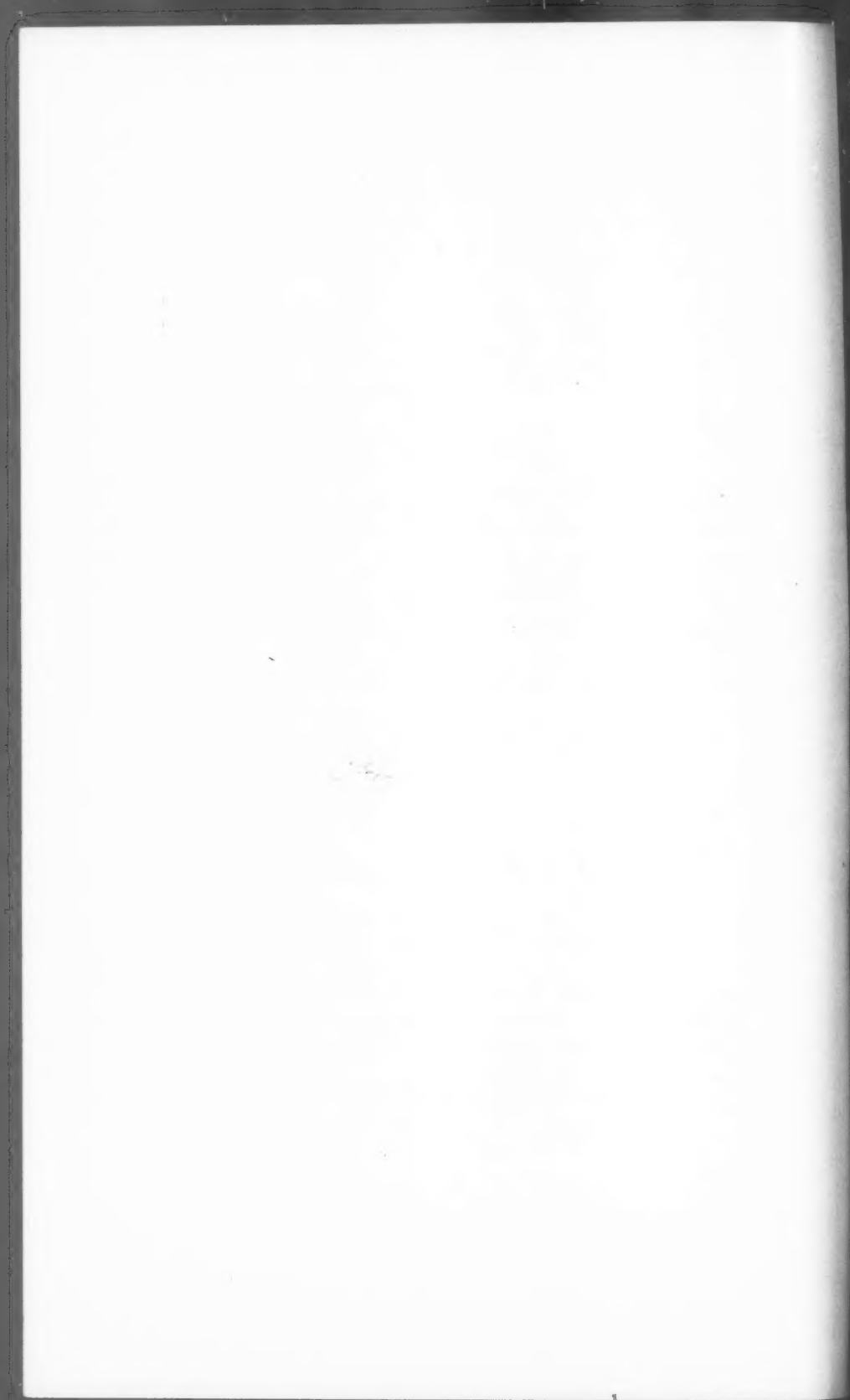
Isolation of Mumps Virus at Autopsy